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SPHACELOMA PERSEAE THE CAUSE OF AVOCADO SCAB [1]

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INTRODUCTION

Scab of avocado (*Persea americana* Mill.), first recognized as a severe disease in avocado nurseries in Florida (*17*),[3] [4] has been more or less prevalent (*4, pp. 65R-66R*) [5] in all avocado-growing regions of the State for several years and is regarded (*4, pp. 65R-66R*) [6] as one of the most important avocado diseases in Florida.

This paper embodies the results of the writer's studies of avocado scab which led to the recognition of its causal fungus as a member of the genus *Sphaceloma* and as a distinct species in this genus (*10*), i.e., *S. perseae* Jenkins (*13*). The field work was conducted in Florida, chiefly at the United States Horticultural Field Laboratory at Orlando, from February to June 1925. It was carried on in conjunction with similar studies of *S. fawcettii* Jenkins, which causes scab of *Citrus* (*9*) and with which *S. perseae* was formerly believed to be identical (*4, p. 48R; 17; 18; 19*).

APPEARANCE OF SCAB LESIONS

The general appearance of scab lesions as observed by the writer at Orlando, Fla., is illustrated by the accompanying plates, except that they do not show lesions on the upper surface of recently expanded leaves. Often "dragon's blood red" [7] in color, these range from punctate areas to spots 2 mm or so in diameter. Lesions are shown on fully expanded but not hardened leaves in plate 1, *F*, and on old leaves of the previous season in plate 1, *G*. These lesions were initiated on the upper leaf surface. Their appearance beneath is shown in plate 1, *A* and *H*, and in plate 2, *B*. Occasionally lesions are arranged in a circle (pl. 2, *B*) as previously recorded in the case of citrus scab (*12, p. 548 and fig. 2, B; 16*). Contrary to the situation in citrus scab, the lower surface of the avocado leaf is less susceptible to infection than the upper surface. On the lower surface initial infection occurs

[1] Received for publication Apr. 25, 1934; issued January 1935. Part of the subject matter of this paper is from a thesis presented to the faculty of the Graduate School of Cornell University, in June 1927, in partial fulfillment of the requirements for the degree of doctor of philosophy.

[2] Thanks are due H. M. Fitzpatrick, L. M. Massey, and A. J. Eames, under whose direction the doctorate work was done. Acknowledgment is also made of the assistance rendered by the late O. F. Burger, H. R. Fulton, the late W. J. Krome, T. Ralph Robinson, J. R. Winston, and others, including the Florida State Plant Board. The field work was conducted in cooperation with the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U.S. Department of Agriculture, and with the University of Florida.

[3] Reference is made by number (italic) to Literature Cited, p. 868.

[4] STEVENS, H. E. AVOCADO SCAB AND ITS CONTROL. 2 pp. January 1932. [Mimeographed.]

[5] FULTON, H. R. DISEASES OF FRUIT AND NUT CROPS IN THE UNITED STATES IN 1926. U.S. Dept. Agr., Bur. Plant Indus. Plant Disease Repter. Sup. 52: 93–104. 1927. [Mimeographed.]

[6] ROBINSON, T. R. AVOCADO CULTURE IN FLORIDA. 13 pp. July 1933. [Mimeographed.]

[7] Names of colors in quotation marks are according to the following authority: RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D.C. 1912.

commonly on veins and midrib (pl. 1, *A*, and pl. 2, *A*). Plate 1, *E*, represents stem lesions on a new shoot, and plate 1, *B*, *C*, and *D*, lesions on fruits of different ages.

The lesions of scab here described and illustrated are generally like those described by Stevens (*17*, *18*). However, he did not refer to lesions on recently expanded leaves, nor to those on midrib and veins on the lower side of the blade.

DEVELOPMENT OF CONIDIAL FRUCTIFICATIONS

On newly formed scab lesions, including those on avocado fruits just set, acervuli of *Sphaceloma perseae* had just ruptured the epidermis and in some cases had produced hyaline biguttulate conidia, like those illustrated in plate 2, *D*. Through continued growth (pl. 3 and pl. 4, *D* and *H*) the conidia and conidiophores formed a dense velvety covering over somewhat older lesions (pl. 1, *A–F*). In mass this conidial growth was generally "dark olive". On the lower leaf surface, however, where it was less abundant than on the upper (pl. 3, *A*), it was commonly "light brownish olive".

When the fruit shown in plate 1, *B*, was about 1 month old, i.e., about the size and age of the fruit shown in plate 1, *D*, the large diseased area, then just becoming russeted, was densely covered with conidial growth of the fungus. This covering gradually weathered away, however, until only an occasional conidiophore was present when the fruit was 4 months old, as illustrated.

The "brown or black" (*18*) "webby" (*17*) fungus growth on avocado scab lesions, formerly believed (*18*) to be of secondary nature, is actually colored conidial growth of *Sphaceloma perseae*, in the opinion of the present writer. The whitish fuzzy growth of the fungus, also previously noted (*18*), evidently corresponds to that produced (*12*, *pp. 551–552*, *fig. 2*, *A*) by *S. fawcettii*.

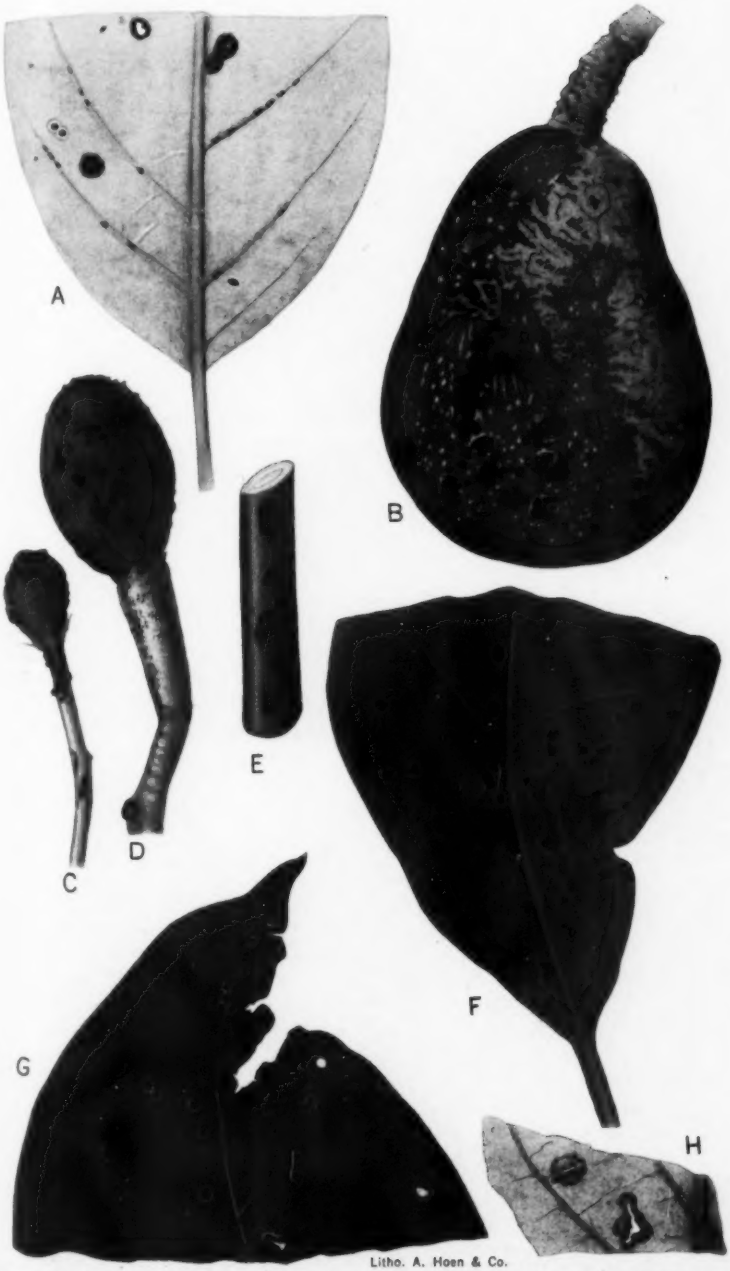
That conidial fructifications of *Sphaceloma perseae* are generally larger or coarser than those of *S. fawcettii* is revealed by comparing the photomicrographs here shown in plates 3 and 4, *D* and *H*, with those of *S. fawcettii* (*12*, *fig. 4*, *A*, *B*, *E*, *F*, *and G*) of practically the same magnification. By actual measurement the conidiophores of *S. perseae* are often 25μ to 50μ and the conidia from 12μ to 20μ in length, while the conidiophores of *S. fawcettii* seldom attain a length of more than 25μ or 30μ, and its conidia are commonly 10μ long, occasionally reaching 16μ. These structures are somewhat more slender in *S. fawcettii* than in *S. perseae*. As the two fungi were viewed in mass at Orlando, the generally "drab" color of *S. fawcettii* contributed to its more delicate appearance. No other fungi of the genus *Sphaceloma* with as prominent conidial growth are known.

CULTURAL STUDIES

SOURCES OF ISOLATIONS

Table 1 lists the sources of the isolations of *Sphaceloma perseae* from Florida made during the course of the present study. These isolations were made both by means of tissue cultures from scab lesions and by platings from conidial growth. Agar media, such as potato-dextrose, Molisch, or glycerin,[8] were employed. The isolation referred to as culture 21 (table 1) consisted of cultivations of the fungus from lesions on different organs of an avocado tree, including leaves, stems, and

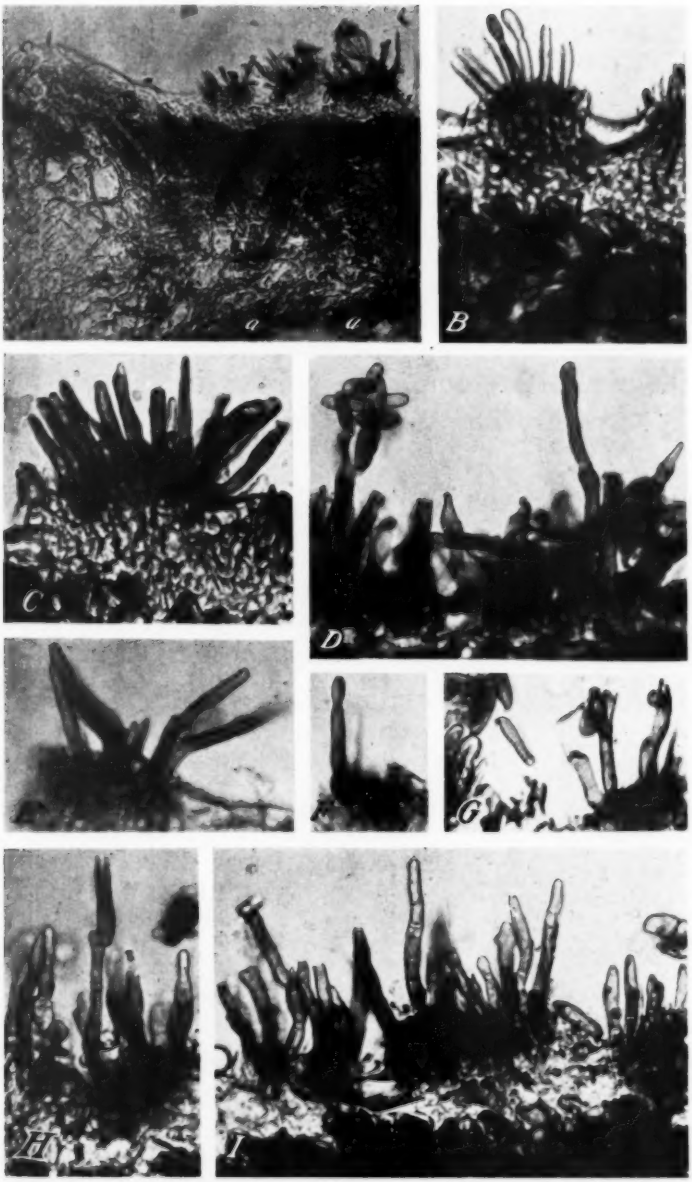
[8] Formula given by Winston (*19*, *p. 9*).



Litho. A. Hoen & Co.

Scab lesions on different organs of avocado: *A*, Lower surface of young leaf; *B*, an approximately 4-month-old fruit; *C*, young fruit and pedicel; *D*, a somewhat older fruit; *E*, young stem; *F*, upper surface of young leaf; *G*, upper, and *H*, lower surface of old leaves. *H*, × about 2; all others ×1. Material from Orlando, Fla., 1925; *B*, collected in June, the other specimens in April. Color drawings by J. Marion Shull.

A and *B*, Lower leaf surface of Fuerte avocado showing scab lesions on (*A*) midrib, and (*B*) between veins in circular arrangement. ×1. *C*, Avocado-scab lesions on veins and midrib of Perfecto leaf artificially inoculated on lower surface with *Sphaceloma perseae*. ×¾. *D*, Hyaline primary conidia of *S. perseae* from culture. ×380. *E*, Infection of Trapp leaf artificially inoculated on upper surface with *S. perseae* from culture. ×1. *F*, Secondary conidia of *S. perseae* produced in culture. ×200. *G* and *H*, Upper (*G*) and lower (*H*) surfaces of Perfecto leaf artificially inoculated on upper surface with *S. perseae* from nature. ×1. *I*, Culture of *S. perseae* from Florida, on wort agar. *J*, Culture of *S. perseae* of the pulvinate type of growth on potato-dextrose agar. *K*, *S. fawcettii* on potato-dextrose agar, showing convolute type of growth in sectional view. ×3.



A, Section of avocado leaf showing conidiophore clumps of *Sphaceloma perseae* on upper surface of blade, and (*a*) bases of similar clumps on lower surface. × about 100. *B–I*, Conidiophores and conidia similar to those shown in *A*. *D* and *G*, From leaf; the others from fruit. × about 600.

fruit of a recent flush of growth and leaves of an old flush. The isolations from the leaf blades were from interveinal lesions and lesions on midrib and veins beneath.

TABLE 1.—*Isolations of Sphaceloma perseae from avocado grown in Florida*

Culture no.	Date of isolation	Source of specimens from which the isolations were made			
		Variety when known, or stock	Locality	Date collected	Collector
20	Mar. 23, 1925	Nursery stock	Rockdale	Mar. 21, 1925	W. J. Krome.
59	Apr. 12, 1925	Perfecto	Orlando	Apr. 11, 1925	A. E. Jenkins.
60	do	do	do	do	Do.
21	Apr. 15, 1925	Fuerte	do	Apr. 15, 1925	Do.
23	May 6, 1925	Seedlings	Vicinity of Lake Hamilton.	May 4, 1925	H. C. Artis.[2]
29	May 8, 1925	Fuerte	Orlando	May 8, 1925	A. E. Jenkins.
30	May 16, 1925	West Indian seedling	Windermere	May 15, 1925	J. R. Springer.[2]
31	do	do	St. Cloud	May 12, 1925	Do.
32	do	Budded stock	Tampa	May 13, 1925	M. R. Brown.[2]
34	May 23, 1925	Perfecto	Orlando	May 26, 1925	A. E. Jenkins.
35	do	do	do	do	Do.
[1] 37	do	do	do	do	Do.
[1] 39	do	Trapp	do	do	Do.
[1] 40	do	do	do	do	Do.
48	June 11, 1925	West Indian seedling	Homestead	May 30, 1925	A. E. Jenkins and W. J. Krome.
49	do	Lula	Winter Haven	May 29, 1925	H. C. Artis.
[1] 54	June 25, 1925	Perfecto	Orlando	June 22, 1925	J. F. Wootten and W. J. Bach.
[1] 55	do	do	do	do	Do.
57	July 3, 1925	Lula	Homestead	June 26, 1925	E. L. Kelley.[2]
88	Apr. 22, 1926		Orlando	Apr. 20, 1925	F. A. Wolf.

[1] Isolation was made in connection with the inoculation experiments reported in table 2.
[2] Inspector of the Florida State Plant Board.

In glycerin agar platings thalli became visible in a week or so. They were light colored at first, but they soon became "Morocco red", or "Morocco red" at the center shading to "Napal red", with the marginal zone light colored and somewhat translucent. Frequently they were glistening or of metallic luster, due to a clear, viscid substance that developed on their surface.

The cultures were practically uniform in appearance, and in all cases the fungus isolated was typical of the genus *Sphaceloma* as previously studied in culture. This was determined by parallel cultural comparisons of thalli from a given isolation and of representative thalli from different sources.

Cultures of the organism on potato-dextrose agar were found to be viable after 9 months, their viability having been determined by transferring the entire culture, then entirely dry, to a fresh agar substrate.

TYPES OF GROWTH

The cultures from Florida were all of the convolute type of growth. A culture isolated from avocado from Grahamstown, Cape Province,[9] however, is of the pulvinate type.[10] Plate 4, *A* to *C* and *E* to *G*, shows reverse, face, and sectional views of these two growth types of *Sphaceloma perseae*. These cultures and that shown in plate 2, *K*, illustrating convolute growth of *S. fawcettii* in section, are from parallel cultures about 2 months old. The pulvinate character of

[9] Contributed by E. M. Doidge, of the Department of Agriculture, Pretoria, Union of South Africa, in 1930, isolated May 20, 1929.
[10] The two types of growth are described in a related paper (*12, pp. 554–555, fig. 5*).

the culture from Cape Province is better shown in the younger culture on potato-dextrose agar illustrated in plate 2, *J*. On wort agar marked cerebriform growth is sometimes produced by the convolute growth type of the fungus (pl. 2, *I*.)

The two types of growth are often of different coloration. For example, 3-weeks-old thalli on potato-dextrose agar slants were black and "gray" for the convolute type, and "pale pinkish cinnamon" for the pulvinate type. In an older set of cultures on this medium, held in the refrigerator for several weeks, the thalli of the convolute type were chiefly "deep colonial buff" where pubescent, and, where glabrous, "Sanford's brown". "Clay color" was the predominating coloration of corresponding cultures of the pulvinate type, although in some parts they were "pale pinkish cinnamon". In all cases the medium had become colored "lime green".

Comparisons of the same growth types reveal that *Sphaceloma perseae* is culturally separable from *S. fawcettii* and other members of the genus *Sphaceloma* that have been cultured. Plate 4, *I* to *P*, represents an early comparison on Molisch agar, made in 1925, when only the five species there represented were known to belong to the same genus of the Fungi Imperfecti, i.e., *Sphaceloma*. Only convolute growth types had then been isolated for *S. perseae* and *Elsinoe ampelina* (DBy.) Shear. *J* and *K* show (*J*) convolute and (*K*) pulvinate types of *S. fawcettii; M* and *N*, those of *E. veneta* (Speg.) Jenkins; and *O* and *P*, those of *S. rosarum* (Pass.) Jenkins.

In plate 4, *Q* to *Z*, corresponding growth types of *Sphaceloma perseae* and *S. fawcettii* are shown on potato-dextrose, Molisch, and carrot-agar media. In the order just named, *Q* and *R* represent their convolute type, and *S* and *T* their pulvinate type on potato-dextrose agar; *U* to *V* their convolute type and *W* and *X* their pulvinate type on Molisch agar; and *Y* and *Z* their pulvinate type on carrot agar.

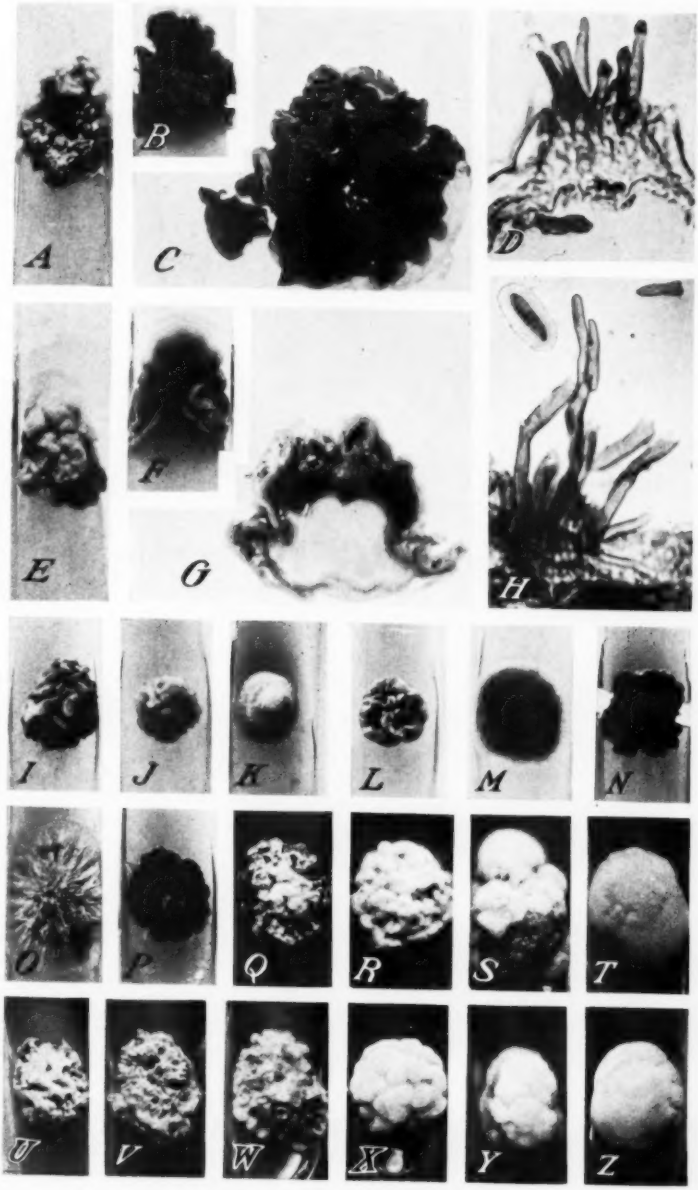
CONIDIAL FORMATION IN CULTURE

At Orlando an olivaceous conidial layer similar to that produced on scab lesions often developed on the surface of Molisch-agar slant cultures of the fungus. Appearing when the thalli were about a week old, it was visible for 5 to 10 days and then ceased to be evident. This conidial development was again present on slant cultures of the fungus on potato-dextrose agar grown at Ithaca, N.Y. (March 1927). On cultures held at constant temperatures of 5°, 7.5°, 10°, 15°, and 20° C., it was present only on those at 20°, the optimal for mycelial growth of the fungus at these temperatures.

As is usual for members of the genus *Sphaceloma*, hyaline conidia were readily obtained in culture by transferring small particles of

EXPLANATORY LEGEND FOR PLATE 4

A–C, Face (*A*), reverse (*B*), and sectional (*C*) views of *Sphaceloma perseae*, convolute type of growth. *A* and *B*, × 1; *C*, × 3. *D*, Young fructifications of *S. perseae* on avocado leaf showing ruptured epidermis. × about 600. *E–G*, Face (*E*), reverse (*F*), and sectional (*G*) views of pulvinate growth type of *S. perseae*. *E* and *F*, × 1; *G*, × 3. *H*, Additional conidiophores and conidia of *S. perseae* from nature. × about 600. *I–P*, Parallel cultural comparison of *S. perseae* from Florida (*I*) with the following cultures: *J* and *K*, *S. fawcettii*, *J* from rough lemon (*Citrus limonia* Osbeck), Florida, isolated by Stevens, 1916, and *K* from Tahiti lime (*C. aurantifolia* Sw.), Florida, isolated by the writer, 1925; *L*, *Elsinoe ampelina* from grape, Florida, isolated by the writer, 1922; *M* and *N*, *E. veneta*, *M* from *Rubus occidentalis* L., Wisconsin, isolated by L. K. Jones, 1922, and *N* from *Rubus neglectus* Pk., Rossiyn, Va., isolated by the writer, 1925; and with *O* and *P*, *S. rosarum* (Pass.) Jenkins, *O* from *Rosa* sp. var. Van Fleet, Ithaca, N.Y., isolated by L. M. Massey, 1923, and *P* from *Rosa* sp. var. Crimson Globe, Ithaca, N.Y., isolated by the writer, 1925. For further explanation see text. *Q–Z*, Parallel cultural comparison of the two growth types of *S. perseae* and *S. fawcettii* on *Q–T*, potato-dextrose, *U–X*, Molisch, and *Y–Z*, carrot-agar media. For further explanation see text.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE.

mycelial cultural growth to a moist atmosphere. The conidia shown in plate 2, *D*, are primary conidia from a 48-hour-old transplanting on the surface of a corn-meal agar plate. The similar conidia shown in plate 2, *F*, are of secondary origin, and presumably were produced during an 18-hour period at the beginning of which a small amount of water was added to a culture corresponding to that shown in plate 2, *D*.

TECHNICAL DESCRIPTION, VARIETIES AFFECTED, AND GEOGRAPHIC RANGE

For convenience in reference the technical description of *Sphaceloma perseae* (*13*), which was based on the present studies, is quoted below:

Lesions generally brown to almost black, up to 3 mm in diam.; conidial fructifications, acervuli at first, with further development constituting sporodochia or more or less free conidiophore tufts, scattered to effuse, in mass, dark olive or light brownish olive, 25μ–70 ± μ in length; conidiophores at first 1–2-celled, often about 12μ high by 2μ–7μ at base, tapering, or acute, to truncate at apex, arising from hyaline intra-epidermal hyphae or from prosenchymatous stroma, palisaded, on rupturing the epidermis increasing in length by continued growth or by conidia remaining *in situ* and developing as a part of the conidiophores, often 25μ to 50μ long, reaching 100μ, more or less divergent, continuous to several septate, straight or geniculate, usually simple, sometimes denticulate, apical region often paler than rest of conidiophore; conidia acrogenous or pleurogenous, at times several produced from the same point, hyaline or colored, clear or granular, spherical to cylindrical, 2μ–30μ×2μ–5μ; hyaline conidia ovoid or oblong-elliptical, often 5μ–8μ×3μ–4μ, sometimes biguttulate, continuous, at least when first formed; elongate colored conidia 1–6-celled, reaching 30μ×3μ–5μ, often 1–2-celled, 12μ–20μ long; conidia sometimes greatly enlarged or swollen, swollen conidia at times muriform; germination by hyaline sprout conidia or by germ tubes, often produced apically or subapically but also laterally.

The perfect or ascomycetous stage of *Sphaceloma perseae* has not been found, although what appeared to be immature asci of the myriangiaceous genus *Elsinoe*, hence possibly this stage, was present on leaf lesions of the current season's growth of avocado collected on May 19, 1925.

In Florida avocado varieties highly susceptible to attack by *Sphaceloma perseae* are Fuerte (*18*) and Lulu,[11] [12] Mexican-Guatemalan hybrids; Trapp (*18*), of the West Indian race; and Taylor (*18*), of the Guatemalan. During the present studies the susceptibility of three additional Guatemalan varieties was also noted. These are Challenge, Perfecto, and Surprise, which, according to Stevens,[13] are not of commercial importance in Florida. During the season of 1933 this authority observed [13] the disease on 12 avocado varieties, half of which are grown in most of the larger plantings of the State.

In Cuba (*3*, *p. 59*) and Puerto Rico (*14*, *p. 31*) native avocado varieties are said to be rarely attacked, and, as mentioned below, they were also not affected in Sao Paulo, Brazil.

The known geographical range of *Sphaceloma perseae* includes Florida, Cuba (*2*, *3*), Puerto Rico (*14*), Haiti,[14] Mexico (*11*), where the avocado grows wild (*15*), Peru (*1*), and the Transvaal (*8*). Carneiro (*5*) stated that avocado scab probably occurs in Brazil. This has proved to be the case, for A. S. Müller, of the Escola Superior de Agricultura, Vicosa, Minas Geraes, Brazil, recently told the

[11] ROBINSON, T. R. See footnote 6.
[12] The races of the avocado varieties here listed are according to Condit (7).
[13] Letter from H. E. Stevens dated Feb. 26, 1934.
[14] Occurrence in Haiti based on specimen from that country intercepted by the U.S. Plant Quarantine and Control Administration, 1930.

present writer that he had observed the scab in severe form on July 1, 1932, in Sao Paulo, on avocados originally from Florida, but not on native varieties. A. Bitancourt, of the Instituto Biologico de Defesa Agricola e Animal, Sao Paulo, Brazil, also noted the occurrence of the avocado scab in Sao Paulo, and sent specimens collected in September 1933.[15]

Thus far the disease has not been found in California,[16] in the Hawaiian Islands (*6*), or in Rhodesia.[17]

INOCULATION EXPERIMENTS

ON AVOCADO

Field inoculation experiments on young unfolding avocado leaves (0.7 to 2 cm in length) of trees growing in the avocado collection at the United States Horticultural Field Laboratory at Orlando were made in the spring of 1925. The cultures of *Sphaceloma fawcettii*, *Elsinoe veneta*, and *E. ampelina* employed as inocula (table 2) were the same as those illustrated in plate 4, *J*, *M*, and *L*, respectively. The sources of other inocula used are given in table 2.

TABLE 2.—*Readings on artificial inoculations with Sphaceloma perseae, S. fawcettii, Elsinoe veneta, and E. ampelina, made on avocado in the field at Orlando, Fla., 1925*

Experiment no. and date of inoculation	Organisms and controls	Avocado variety	Leaf surface inoculated	Number of leaves or leaf clusters inoculated	Number of leaves or leaf clusters infected [1]	Degree of infection [2]	Lesions initiated mostly or entirely on leaf surface indicated
1, Mar. 16	*S. fawcettii*, culture 8	Perfecto	Lower [3]	24	9	+−−	Upper.
	E. veneta, culture 9			23	[4] 2	+−−to++	
	E. ampelina, culture 12.			15	[4] 10	+to++	
	Controls			21	0	0	
2, Apr. 23	*S. perseae*, culture 20	Trapp and Perfecto.	do	16	[4 5] 10	+−−to+	Lower.
	S. fawcettii, culture 8			19	1	+−−[6]	
	Controls			21	1	+−−[6]	
3, May 4	*S. perseae*, culture 20	Trapp	Upper	13	[4] 12	+to++	Upper.
	Controls			13	2	+to++	
4, May 5	*S. perseae*, cultures 20 and 60.	do	do.[7]	14	[4] 13	+to++	Do.
	Controls			9	0	0	
5, May 11	*S. perseae*, from nature.	Perfecto	do	10	10	+to++	Do.
	Controls			9	0	0	
6, May 20	*S. perseae*, from nature.	do	do	7	[4] 7	+to+++	Do.
	S. fawcettii from nature.			11	9	+to++	
	Controls			12	[4] 9	+−−to+	
7, May 24	*S. perseae*, culture 20	do	do	12	12	+to+++	Do.
	S. fawcettii, culture 8			6	6	+−−	
	Controls			7	5	+−−	

[1] All lesions typical of avocado scab, i.e., all infection by *S. perseae* and none from the cross inoculations.
[2] The degree of infection is indicated by the following symbols: +++, very heavy; ++, heavy; +, moderate; +−, light; +−−, very light; 0, none.
[3] Chiefly lower surface.
[4] Isolations were made and all proved to be of *S. perseae*, as indicated in table 1.
[5] Isolations from vein lesions on 2 different leaves.
[6] Single lesion on vein, lower leaf surface.
[7] One leaf recorded as inoculated on both surfaces of the blade became infected on both surfaces.

[15] Müller has written, in a letter dated June 18, 1934, that on this date he found at Viscosa a relatively small amount of infection " in the college nurseries of avocados, on 2 year-old seedlings of the variety 'Antillhana' . . . and on 5 Winslowan grafts, 6 months old."
[16] Letter from William T. Horne, of the University of California, dated Nov. 22, 1933.
[17] Letter from J. C. F. Hopkins, Department of Agriculture, Salisbury, Southern Rhodesia, dated Apr. 10, 1934. Based on a literature report not available at present for verification, it was stated previously (*13*) that *Sphaceloma perseae* occurs in Rhodesia.

The cultural inocula consisted of growth from 10 days to several weeks old on test-tube slants of potato-dextrose, Molisch, or glycerin agar media. Parallel cultures on the same substrate were used for a given experiment. In preparing the inocula for application, that for each fungus was removed from the agar surfaces, macerated with a glass rod or cut in small pieces with a scalpel, and then transferred to wet absorbent-cotton pads. Inocula from nature, consisting of particles of lesions bearing conidial fructifications, were transferred to small pieces of wet filter paper. In the first four tests the cultural inocula were applied as soon as prepared and when examined at the time of application were sterile. It is presumed, however, that the wet cotton pads provided favorable conditions for the prompt formation of hyaline conidia, as occurred in the corn-meal agar cultures already described (p. 863). The inoculum for each of the remaining three tests was arranged in a Petri dish and then held for 24 hours in an ice-cooled refrigerator before being applied. At the end of this period hyaline conidia had sprouted from the colored conidia contained in the inocula from nature.

In each experiment the leaves inoculated were grouped on parts of trees showing no infection at the beginning of the experiment. After inoculation they were covered with waxed-paper hoods secured around the avocado stem with rubber bands or string. In inoculation test 7 (table 2) the wrappings were removed after 32 hours, and in the other inoculation tests after 48 hours. Thereafter the inoculated growth and the corresponding growth on the same part of the trees were examined daily, or practically so, for evidence of infection.

In experiment 1, made before *Sphaceloma perseae* had been isolated and found to be distinct from *S. fawcettii*, each cotton or plaster, i.e., each individual inoculation, was wrapped around the outside of a cluster of new leaves at the end of a single shoot or branch. The inocula here naturally came in contact chiefly with the lower or dorsal leaf surfaces. In subsequent tests a single leaf constituted an individual inoculation. The lower surface was inoculated in experiment 2, but before results were obtained from this experiment it had become evident that the upper side of the avocado leaf is more highly susceptible to infection by *S. perseae* than the lower. Subsequent inoculations were therefore made on the upper side of the leaf surface.

ON CITRUS

The first two inoculation experiments on avocado (table 2) included parallel inoculations on citrus. Young fruits of lemon and of grapefruit (*C. grandis* Osbeck) were employed in the first experiment and young leaves of grapefruit and sour Rangpur lime (*C. aurantifolia* Sw.) in the second. Practically no data were obtained from these experiments, for nearly all the fruit became severely affected by citrus scab as a result of natural inoculation by *S. fawcettii*, and soon dropped. Essentially the same situation obtained in the other experiments, although from 3 to 15 of the leaves inoculated with a given fungus or used as controls remained attached at the end of the experiment. It so happened that there was no citrus scab on the 3 grapefruit leaves inoculated with *S. perseae* that remained at the close of the experiment. These leaves were otherwise healthy.

Additional inoculations were made on unfolding leaves of grapefruit, lemon, and Cuban shaddock under greenhouse conditions.

These gave infection with *Sphaceloma fawcettii*, as previously reported (*12*), but none with *S. perseae* (table 3). Although not as extensive as the cross inoculations on avocado with *S. fawcettii*, because of the limited amount of new growth available when the inoculations were made, these experiments gave no suggestion that *S. perseae* is pathogenic on citrus.

TABLE 3.—*Greenhouse inoculation tests on citrus with Sphaceloma perseae and S. fawcettii*

Date of inoculation	Kind of citrus	Inocula and controls		Number of leaves inoculated	Number of leaves infected	Location of greenhouse
		Species	Culture no.			
May 22, 1926	Cuban shaddock (*Citrus* sp. hybrid, no. 11893) [1]	*S. perseae*	21	7	0	Arlington Experiment Farm, Rosslyn, Va.
		S. fawcettii	8	7	4	
		Controls		7	0	
Do	Grapefruit seedling (*Citrus grandis*)	*S. perseae*	21	2	0	Do.
		S. fawcettii	8	2	1	
		Controls		2	0	
Mar. 14, 1927	Rough lemon (*Citrus limonia*, no. 7610)	*S. perseae*	21	4	0	Cornell University, Ithaca, N.Y.
		S. fawcettii	8	4	2	
		Controls		4	0	

[1] The serial numbers cited are those of crop physiology and breeding, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, by which the plants inoculated were contributed.

DISCUSSION OF RESULTS

Data resulting from the inoculation experiments summarized in table 2 yielded no evidence that *Sphaceloma fawcettii*, *Elsinoe veneta*, or *E. ampelina* (experiment 1) are pathogenic on avocado. The lesions were initiated mostly on the upper leaf surface, whereas the artificial inocula were applied chiefly on the lower leaf surface. This orientation of the lesions was evidence against infection having resulted from the cross inoculations, as well as the fact that the lesions themselves could be diagnosed as of avocado scab. In the other experiments infection present on the leaves inoculated with *S. fawcettii* and on the controls could again be construed only as infection from natural inoculation by *S. perseae*. The degree of infection on all this growth agreed in general with that on nearby uninoculated leaves of the same description as those inoculated.

In experiment 2 there was good infection from the artificial inoculations with *Sphaceloma perseae*, considering the fact that the lower or less susceptible leaf surface was inoculated. Infection from natural inoculation was practically negligible.

Experiments 3, 4, and 5, in which only *Sphaceloma perseae* was employed and the upper side of the leaf was inoculated, comprised a total of 37 artificially inoculated leaves. All but 2 of these became infected. There was again almost no infection as a result of natural inoculation by *S. perseae*. All or most of the infection on the artificially inoculated growth obtaining in these three experiments, as well as in experiment 2, certainly resulted from the artificial inocula. The degree of infection on artificially inoculated leaves in experiments 3, 4, and 5 was greater than on those in experiment 2, because of the fact that the upper or more susceptible leaf surface was inoculated.

It was anticipated that in experiment 6 there would be more or less infection by *Sphaceloma perseae* as a result of natural inoculation,

because there was a slight amount of infection in the general region of the inoculated leaves when the inoculations were made. Moreover, some of the leaves available for inoculation were older than those ordinarily chosen for this purpose and might already have been inoculated naturally. This proved to be the case, for lesions had become visible on some of these leaves at the time the waxed-paper hoods were removed, or before there was time for the appearance of symptoms from the artificial inoculations. In both this experiment and experiment 7, however, it was apparent that the increased amount of infection on growth artificially inoculated with *S. perseae* was attributable to the positive results from the artificial inoculum.

The heaviest infection from the artificial inoculations occurred in experiment 7, wherein leaves were thoroughly wet from a pouring rain when inoculated. The lesions were unusually large, some reaching 3 mm in diameter, and some of the leaves were considerably distorted.

In the inoculation experiments the incubation period of *Sphaceloma perseae* was about 7 days. Thus in experiment 5 lesions were first visible as minute reddish points on the seventh day after inoculation. By the tenth day they had increased to a diameter of 0.7 mm and were "dragon's blood red" above and "light lobelia violet" beneath. A week later a yellowish border had developed around them and most of them had ruptured the epidermis. In the rupturing process the epidermis became blistered, then raised at the center of the blistered area and there perforated as though an insect had emerged. The circular perforation later expanded, exposing the necrotic leaf tissue beneath (pl. 2, *H*). The photographs of the leaf here shown were made after the leaf had matured and the bright colorations of the lesions had disappeared.

The inoculation experiments serve to demonstrate that the dorsal or lower surface of the avocado leaf is less susceptible to infection by *Sphaceloma perseae* than the upper or ventral surface and that on the dorsal surface the midrib and veins are more susceptible than interveinal regions. Thus the leaf from experiment 2, represented in plate 2, *C*, shows infection on midrib and veins beneath as a result of inoculating the lower side of the blade, and the leaves from experiments 4 and 5, represented in plate 2, *E* and *G*, show severe infection from inoculations on the upper side of the blade. Plate 2, *E*, also shows infection from cultural inoculum and plate 2, *G*, that from inoculum from nature. The lesions on the leaf shown in plate 2, *G*, outline in part the filter paper on which the inoculum was placed.

The results of the parallel inoculations on citrus and on avocado with *Sphaceloma perseae* and *S. fawcettii* are consistent with each other, since in the inoculations on avocado the results with *S. perseae* were positive and those with *S. fawcettii* negative, whereas the reverse was true when the two fungi were inoculated on citrus. The results of the inoculations on avocado with the two fungi also agree with those of Stevens (*17*). However, both he and Winston (*19, pp. 22–26*) reported infection of citrus by *S. perseae*. Since Winston's work was done in the field, where some infection by *S. fawcettii* was known to be present, he might have mistaken the infection observed for positive results from artificial inoculations which he made with *S. perseae*. Absence of infection of avocado by *S. fawcettii* in the writer's inoculation experiments accords with the fact that in the whole course of her

studies of avocado scab only *S. perseae* was ever found growing on the lesions and no other species was ever isolated from them.

SUMMARY

This paper presents the results of an investigation of avocado scab, on the basis of which its causal fungus was recently described as *Sphaceloma perseae*.

This organism is compared morphologically and culturally with *S. fawcettii* and is shown to be distinct from this fungus, with which it was formerly confused.

In Florida, the avocado varieties Challenge, Perfecto, and Surprise, of the Guatemalan race, are shown to be susceptible to attack by *S. perseae*, in addition to the varieties previously reported as affected, namely, Fuerte and Lulu, Mexican-Guatemalan hybrids, and Trapp and Taylor, of the West Indian and Guatemalan races, respectively.

The geographic range of the fungus is shown to include Florida, Cuba, Puerto Rico, Haiti, Mexico, Peru, Brazil, and the Union of South Africa.

In parallel inoculation experiments with *S. perseae* and *S. fawcettii* infection was obtained on citrus only with *S. fawcettii* and on avocado only with *S. perseae*.

LITERATURE CITED

(1) Abbott, E. V.
1929. diseases of economic plants in peru. Phytopathology 19: 645–656.

(2) Bruner, S. C.
1921. lista preliminar de las enfermedades de las plantas de importancia economic para cuba. Cuba Estac. Expt. Agron. Informe 1918–19, 1919–20: 725–763, illus.

(3) ———
1931. aguacate (persea gratissima gaertn.). Cuba Estac. Expt. Agron., Dept. Ent. y Fitopatologia Informe 1929–30: 58–59.

(4) Burger, O. F.
1922–23. avocado. Fla. Agr. Expt. Sta. Ann. Repts. 1922: 48R; 1923: 65R–66R.

(5) Carneiro, J. G.
1932. as molestias mais graves do abacateiro o seus tratamentos. Chacaras e Quintaes 46: 423–426, illus.

(6) Carpenter, C. W.
1931. diseases of the avocado. Hawaii. Planters' Rec. 35: 31–37, illus.

(7) Condit, I. J.
1931. check list of avocado varieties. Calif. Avocado Assoc. Year Book 1931: 11–22.

(8) Doidge, E. M., and Bottomley, A. M.
1931. a revised list of plant diseases occurring in south africa. 78 pp. Pretoria. (So. Africa [Dept. Agr.] Bot. Survey Mem. 11.)

(9) Jenkins, A. E.
1925. citrus scab fungus. Phytopathology 15: [99]–104, illus.

(10) ———
1925. the avocado scab organism. (Phytopathological note) Phytopathology 15: 807.

(11) ———
1930. additional data on the distribution of two species of sphaceloma. (Phytopathological note) Phytopathology 20: 450.

(12) ———
1931. development of the citrus-scab organism, sphaceloma fawcettii. Jour. Agr. Research 42: 545–558, illus.

(13) JENKINS, A. E.
1934. A SPECIES OF SPHACELOMA ON AVOCADO. Phytopathology 24. 84–85.
(14) LÓPEZ DOMÍNGUEZ, F.
1929. AVOCADO. P. R. Insular Expt. Sta. Dept. Agr. & Labor Ann. Rept. 1926–27: 31.
(15) POPENOE, W.
1920. MANUAL OF TROPICAL AND SUBTROPICAL FRUITS, EXCLUDING THE BANANA, COCONUT, PINEAPPLE, CITRUS FRUITS, OLIVE, AND FIG. 474 pp., illus. New York.
(16) SPEGAZZINI, C.
1920. SOBRE ALGUNAS ENFERMEDADES Y HONGOS QUE AFECTAN LAS PLANTAS DE 'AGRIOS' EN EL PARAGUAY. An. Soc. Cient. Argentina 90: [155]–188, illus.
(17) STEVENS, H. E.
1918. AVOCADO SCAB. Fla. Agr. Expt. Sta. Press. Bull. 289, [2] pp. (Reprinted 1919.)
(18) ———
1922. AVOCADO DISEASES. Fla. Agr. Expt. Sta. Bull. 161, 23 pp., illus.
(19) WINSTON, J. R.
1923. CITRUS SCAB: ITS CAUSE AND CONTROL. U.S. Dept. Agr. Bull. 1118, 39 pp., illus.

EFFECTS ON WHEAT PLANTS OF OPHIOBOLUS GRAMINIS AT DIFFERENT LEVELS IN THE SOIL[1]

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INTRODUCTION

It is well known that *Ophiobolus graminis* Sacc. may live for considerable periods in the surface layers of the soil in infested fields, but little is known regarding the vertical distribution of the fungus in the soil of such fields or its effects on wheat plants when it is present in certain soil layers only. Such information is important in devising control measures based on soil management. In order to secure data relating to this problem, experiments were conducted at Manhattan, Kans., in 1925–26, 1926–27, and 1927–28. During the first season (1925–26) the tests were directed toward determining the depth of the fungus in naturally infested fields. During 1926–27 and 1927–28 experiments in both the greenhouse and the field were conducted to determine the effects on wheat plants of *O. graminis* at various levels in the soil.

METHODS

Ophiobolus graminis cannot be readily detected in infested soil either by visual inspection or by plating the soil on artificial culture media. A more satisfactory method is to grow susceptible wheat plants in suspected samples of soil under conditions favorable for infection by the fungus and adequately guarded against contamination from other sources.

In a number of fields near Manhattan, Kans., naturally infested with *Ophiobolus graminis*, soil samples were secured from 1- or 2-inch layers at different successive depths. In securing the samples, care was taken that no sample was contaminated by soil from other layers. The presence or absence of the fungus in the different soil samples was determined by placing them in 6-inch flowerpots in the greenhouse, sowing Kanred wheat in them, and noting whether the disease developed on the plants. In each series, under the same conditions, wheat plants were grown as controls in noninfested soil of the same type.

In order to determine what effects the fungus had on wheat plants when it occurred in the soil at various vertical distances from the seed, inoculum was placed at various distances above and below the seed at the time of planting. The positions and distances ordinarily used were 1 inch above the seed, immediately above, immediately below, and 1, 2, 3, 4, 5, and 6 inches below the seed, respectively. The

[1] Received for publication May 29, 1934; issued January, 1935. Investigations conducted cooperatively by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Kansas Agricultural Experiment Station. Contribution no. 326 of the Department of Botany and Plant Pathology, Kansas State College of Agriculture and Applied Science.

inoculum was placed at these different levels both in separate pots in the greenhouse and in different trenches in the field. The inoculum, with few exceptions, consisted of a 1-inch layer of naturally or artificially infested soil. All surrounding soil was free from *Ophiobolus graminis* and therefore did not require sterilization. In the instances where the inoculum was 1 inch above the seed, immediately above, or

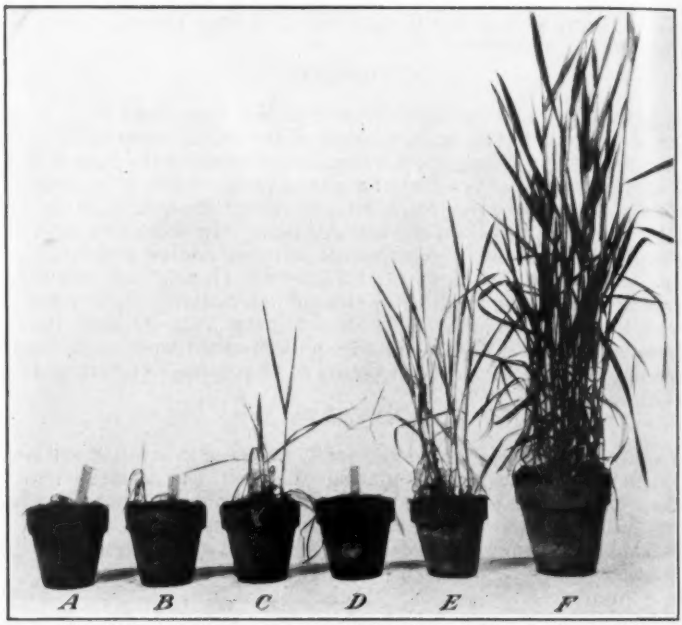

FIGURE 1.—Kanred wheat plants grown in pots containing soil from different levels in a field naturally infested with *Ophiobolus graminis*, and in noninfested soil. *A*, Soil from surface 2-inch layer; all plants killed by the fungus. *B*, Soil from 2- to 4-inch layer; all plants killed. *C*, Soil from 4- to 6-inch layer; almost all plants killed. *D*, Soil from 6- to 8-inch layer; all plants killed. *E*, Soil from 8- to 10-inch layer; plants killed or stunted. *F*, Soil noninfested; plants healthy.

just below it, the seed was sown 2 inches deep. In other cases it was sown 1 inch deep.

At the end of different periods, some of each group of plants were carefully removed from the soil and the condition of the above- and below-ground parts noted.

DEPTH OF OPHIOBOLUS GRAMINIS IN NATURALLY INFESTED FIELD SOIL

Tests were made to determine the depth of *Ophiobolus graminis* in several widely separated fields in the vicinity of Manhattan (fig. 1) during the winter of 1925–26. The results showed the fungus to be present from the surface to a depth of 10 inches. Soil from greater depths was not examined at that time.

Further studies made during the two following seasons showed that the fungus occurred from the surface to a depth of 15 inches. The severity of the disease was somewhat less on wheat grown in the soil

from 13 to 15 inches than from nearer the surface. It may, therefore, be concluded that all underground parts of wheat plants from the surface to 15 inches deep may be in contact with the fungus.

INFECTION EXPERIMENTS IN THE GREENHOUSE

Two kinds of experiments were conducted in the greenhouse, one in which the inoculum consisted of the fungus grown on artificial cul-

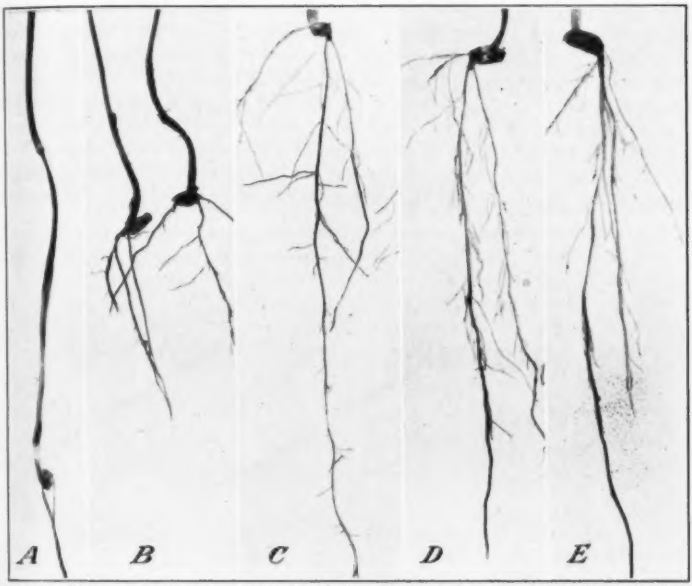

FIGURE 2.—Crowns and primary roots of 26-day-old Kanred wheat plants grown in noninfested soil artificially inoculated at planting time with a culture of *Ophiobolus graminis* placed at different levels above or below the seed. *A*, Inoculum 1 inch above seed; lesion on subcrown internode at level of inoculum only, and not yet advanced downward to seed. *B*, Inoculum immediately below seed; all primary roots diseased and infection advanced upward on coleoptile and subcrown internode. *C*, Inoculum 2 inches below seed; lesions on roots at level of inoculum and extending upward almost to seed and also downward to some extent, the lower portion of diseased roots showing no lesions. *D*, Inoculum 4 inches below seed; lesions on roots at level of inoculum and extending upward and downward to some extent. *E*, Inoculum 6 inches below seed; lesions at level of inoculum and extending upward considerably.

ture media and the other in which the inoculum consisted of naturally infested soil.

The medium used for the artificial cultures consisted of a mixture of equal parts of unground barley and oat kernels moistened and sterilized in Erlenmeyer flasks. *Ophiobolus graminis* was allowed to grow thoroughly through the medium, and the inoculum was then prepared by mixing these cultures with sterilized soil, in the ratio of 1 part of the barley-oat mixture to 4 parts of soil.

ARTIFICIAL CULTURES AS INOCULUM

In one of the experiments in which artificial cultures were used as inoculum the plants were grown in galvanized-iron cans 8 inches in diameter and 12 inches deep, and in the other in cans 3 inches in

diameter and 6 inches deep. The soil in which the plants were grown was maintained at a temperature of 16° C., but the greenhouse air temperatures to which the tops of the plants were exposed were somewhat above this. The soil was held at a moisture content of about 75 percent of the water-holding capacity. These conditions have been found favorable for infection.[2]

In the 8-inch cans, the 1-inch layers of inoculum were placed in the different cans 1 inch above, immediately below, and 2, 4, and 6 inches below the seed, respectively. In the 3-inch cans, the layers of inoculum were placed 1 inch above the seed in 25 cans and immediately below it in 25 others. After different intervals, plants grown in some of the cans were removed and observations recorded. The observations made on the plants grown in the large cans were general, while those on the plants in the small ones were more detailed, especially with respect to the position of the lesions caused by the fungus.

In the 8-inch cans, some of the plants in each group were examined 26 days after planting and the rest 47 days after planting. Examina-

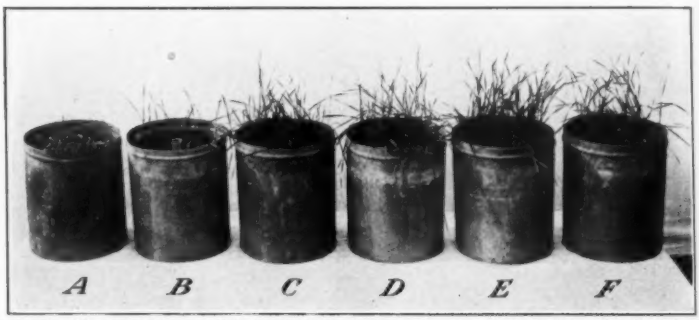

FIGURE 3.—Aboveground parts of 47-day-old Kanred wheat plants grown in noninfested soil artificially inoculated at planting time with a culture of *Ophiobolus graminis* placed at different levels above and below the seed: *A*, 1 inch above the seed; *B*, immediately below the seed; *C*, 2 inches below the seed; *D*, 4 inches below the seed; *E*, 6 inches below the seed; *F*, noninfested soil without inoculum.

tions at the end of 26 days showed the plants to be attacked at all positions of the inoculum, and in some cases lesions appeared nearly an inch from the original point of infection (fig. 2). In the cans in which the inoculum had been placed 1 inch above the seed, immediately below the seed, and 2 inches below it, the aboveground parts of the plants were stunted and yellowed. In the cans in which it had been placed at other positions, no injury was apparent. The injury was progressively less the greater the distance of the inoculum from the seed.

The injury to roots, crowns, and leaves was much greater 47 days after planting than 26 days after planting (fig. 3). A large number of the plants had died. In many cases, lesions appeared as far as 3 inches from the original source of infection. Where the inoculum was 2 inches or less from the seed, secondary roots had formed in a very few cases only. At greater distances, good secondary root formation had occurred. Examples were numerous in which secondary roots had not developed on those plants with badly infected crowns. Injury was not noticeable on the aboveground parts when the inocu-

[2] MCKINNEY, H. H., and DAVIS, R. J. INFLUENCE OF SOIL TEMPERATURE AND MOISTURE ON INFECTION OF YOUNG WHEAT PLANTS BY OPHIOBOLUS GRAMINIS. Jour. Agr. Research 31: 827–840, illus. 1925.

lum was 4 or 6 inches below the seed. At no time in the later history of the plants, when the inoculum was at those distances below the seed, did yellowing, wilting, or stunting show on the aboveground parts. Infection on the roots did not extend to the seed or crown and the plants came to maturity with well-filled heads.

Plants grown in the 3-inch cans where the inoculum was 1 inch above the seed were examined at the end of 9, 20, and 36 days, respectively, after planting, to determine the points of infection and the distances the lesions had extended from the original points of infection. It should be borne in mind that the seed in the 3-inch cans in all cases was sown 2 inches deep. Accordingly, the first inch of soil above the seed was noninfested and the next inch, which extended to the surface, contained *Ophiobolus graminis*.

The depth of crown formation was somewhat variable. Only those plants that had formed their crowns at approximately 1 inch above the seed were used in the readings. The aboveground portions of the plants were not considered. The results obtained where the inoculum had been placed 1 inch above the seed are given in table 1. These results show that at the end of 9 days infection had taken place on only the subcrown internode and that the maximum distance the lesion had extended downward was 1 inch. Secondary roots had not formed at that time. At the end of 20 days, lesions were present on the subcrown internodes and primary roots and had extended, in many instances, as far as the seed and, in some cases, to the primary roots. The greatest total distance was 2.06 inches. At that time, secondary roots had begun to form, but none of them was diseased. At the end of 36 days, lesions extended to the seed and in many cases down both the primary and secondary roots. The greatest total distance that the lesions had covered on the subcrown internode and primary roots was 2.57 inches. Nearly all of the primary roots were diseased, but a large percentage of the secondary roots, especially those formed on the upper parts of the crown, were healthy.

At the end of 36 days after planting, it was noted that where the inoculum had been placed immediately below the seed the infection of the subcrown internode and of the primary and secondary roots was substantially the same as where the inoculum had been placed 1 inch above the seed. The lesions had extended upward in the former case only 0.27 inch less than they had extended downward in the latter. Evidently gravitational water did not have much effect on transporting the fungus through the soil.

TABLE 1.—*Advance downward of Ophiobolus graminis on different organs of Kanred wheat plants, at different periods after planting, from a 1-inch layer of artificial inoculum placed 1 inch above the seed at time of planting*

Plant organ	Advance downward after planting					
	9 days		20 days		36 days	
	Maximum	Average	Maximum	Average	Maximum	Average
	Inches	*Inches*	*Inches*	*Inches*	*Inches*	*Inches*
Subcrown internode	1.00	0.47	1.00	0.90	1.00	1.00
Primary roots	.00	.00	1.06	.35	1.57	.95
Secondary roots	[1]	[1]	.00	.00	1.57	.92

[1] No secondary roots present.

The fact that in both cases a large proportion of the secondary roots escaped injury whereas most of the primary roots were infected may possibly have been due in part to the shorter time of exposure of the secondary roots, as they are formed considerably later than the primary roots. It was observed also that whereas the primary roots are formed almost simultaneously, the secondary roots do not all form at once, there being a considerable interval between the formation of the earliest and the latest. Those formed later are higher on the crown and hence appear less likely to become infected. This may be of some significance, since if the crown, which is the source of the secondary

FIGURE 4.—Kanred wheat plant severely attacked by *Ophiobolus graminis*, which had advanced from the subcrown internode into the crown and had killed all but the uppermost secondary root. Healthy control plants of same age were heading.

roots, does not become too badly invaded, these later-formed secondary roots enable the plants either to recover or to persist much longer than otherwise (fig. 4). This is especially true if the point of attack is some distance from the crown and some time is therefore required for the fungus to advance to the crown.

NATURALLY INFESTED SOIL AS INOCULUM

Three experiments were conducted with naturally infested soil, all in pots in the greenhouse. In experiment 1, conducted in 1926–27, and in experiment 2, conducted in 1927–28, 1-inch layers of naturally infested soil were placed in the pots in the positions desired. In exper-

iment 3, conducted in 1927–28, the infested soil, instead of being placed in 1-inch layers, was used to fill the pots to different levels. The rest of the soil used in these pots, in all three experiments, was of the same type but noninfested. Plants were also grown in pots with infested soil throughout and in pots with noninfested soil throughout for comparison. All the pots were kept under comparable conditions in the greenhouse and with temperature and soil moisture favorable for the development of the disease, as previously indicated. The height of the plants, the percentage of plants headed and killed, the percentage of diseased culms, crowns, and roots, and the advance of the fungus on the roots and culms were recorded at the time of full heading. The data are given in table 2.

Conditions were favorable for the growth of the fungus, as is shown by the fact that in the control pots in which only infested soil was used none of the plants headed, and 100 percent of the plants were killed in experiment 1 and 95 percent were killed in experiments 2 and 3, whereas none of the controls grown in noninfested soil was infected.

TABLE 2.—*Severity of infection by Ophiobolus graminis on Kanred wheat plants grown in the greenhouse in pots inoculated* [1] *at planting time with naturally infested field soil, Manhattan, Kans., 1926–27 and 1927–28*

Soil treatment and experiment no.	Total plants studied	Average height of plants	Plants		Diseased			Advance of the fungus in roots and culms			
								Upward		Downward	
			Headed	Dead	Culms	Crowns	Roots	Maximum	Average	Maximum	Average
Infested soil:	*Number*	*Inches*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Inches*	*Inches*	*Inches*	*Inches*
Just above seed:											
Experiment 1	128	14.5	0.4	0.0						1.57	1.06
Just below seed:											
Experiment 1	83	9.5	.0	49.5						2.36	.94
Experiment 2	68	25.0	43.0	49.0	80.0	100.0	96.0	1.96	0.88	4.51	2.91
Experiment 3	51	6.7	.0	69.4	92.4	100.0	100.0	.78	.40		
1 inch below seed:											
Experiment 1	69	12.2	9.0	44.0				2.20	1.02		
Experiment 2	62	22.5	44.5	39.5	93.0	100.0	100.0	2.37	1.77		
Experiment 3	61	5.7	.0	86.5	96.1	100.0	99.5	2.18	1.47		
2 inches below seed:											
Experiment 1	112	18.5	25.5	.0				2.16	1.85		
Experiment 2	84	16.5	12.6	47.2	50.0	52.5	51.0	3.18	2.85		
Experiment 3	71	8.0	.0	92.2	100.0	100.0	100.0	3.77	2.98		
3 inches below seed:											
Experiment 1	105	16.8	21.0	21.5				3.74	3.07		
Experiment 2	108	20.5	43.0	31.0	50.0	61.8	65.0	4.18	3.11		
Experiment 3	85	18.0	17.1	.0	12.5	95.0	57.0	3.78	2.21		
4 inches below seed:											
Experiment 1	241	23.8	47.0	.0				4.48	2.59		
Experiment 2	102	20.5	59.7	8.0	31.2	81.8	68.5	4.78	4.41		
Experiment 3	114	25.0	74.4	.9	35.0	100.0	85.5	4.98	4.08		
5 inches below seed:											
Experiment 1	138	20.3	26.0	.0				5.35	3.81		
Experiment 2	281	23.5	75.9	.0	40.4	87.0	73.3	5.91	5.41		
Experiment 3	139	24.0	69.8	.0	2.5	100.0	70.0	5.19	4.41		
Infested soil only:											
Experiment 1	33	7.5	.0	100.0							
Experiments 2 and 3	60	8.5	.0	95.0	100.0	100.0	100.0				
Noninfested soil only:											
Experiment 1	60	26.0	40.0	.0							
Experiments 2 and 3	80	26.0	68.4	.0	.0	.0	.0				

[1] The soil was inoculated in experiments 1 (1926–27) and 2 (1927–28) by placing 1-inch layers of naturally infested soil at different levels in the pots, above or below the seed; in experiment 3 (1927–28) by filling the pots with naturally infested soil to within different distances below the seed.

It may be noted that, in general, although the results were irregular, the height of the plants and the percentages of the plants that headed increased with the distance of the inoculum from the seed. Conversely, as would be expected, the percentages of dead plants and diseased culms were greater the nearer the inoculum was to the seed. There were also more dead plants where the infested soil was present in quantity (experiment 3) than where there was only a 1-inch layer, except at those levels where the inoculum was at a distance of 3 inches or more below the seed; at such low levels differences were not consistent. In experiment 1 the absence of dead plants where the inoculum was 2 inches below the seed was unexpected, particularly in view of the fact that, in the same experiment, where the inoculum was 3 inches below the seed, 21.5 percent of the plants were killed. In this connection, it should be noted that in noninfested soil only 40 percent of the plants in experiment 1 and only 68.4 percent

FIGURE 5.—Kanred wheat plants grown in pots containing only naturally infested soil or noninfested soil with 1-inch layers of naturally infested soil at different levels below the seed: *A*, Infested soil throughout; *B*, infested soil immediately below seed; *C*, infested soil 1 inch below seed; *D*, infested soil 2 inches below seed; *E*, infested soil 3 inches below seed; *F*, infested soil 4 inches below seed; *G*, infested soil 5 inches below seed; *H*, noninfested soil throughout.

in experiments 2 and 3 headed. The reason for these irregularities is not known, but the relatively high greenhouse temperatures may have been a factor, as it is well known that winter wheat requires low temperatures during early growth for normal heading. The limited opportunities for root development also may have been a factor.

Data on diseased culms, crowns, and roots were taken only on experiments 2 and 3. While these data also are rather irregular, it will be noted that the percentages of diseased culms tend to be higher the nearer the inoculum was to the seed and that the percentages of diseased crowns and roots are high regardless of the depth at which the inoculum was placed. In most cases an appreciable number of the culms were diseased, even when the inoculum was 4 or 5 inches below the seed.

In most cases the percentages of diseased crowns are similar to those of diseased roots, apparently indicating that if the roots became

diseased to any considerable extent the infection advanced upward to the crown. This is supported by the detailed data on the advance of the fungus in the roots and culms. In all cases the maximum distance of advance exceeded the distance of the inoculum from the seed, and the average distance of advance approached very closely the distance of the inoculum from the seed.

The maximum distance that the lesions extended upward, whatever the depth of the inoculum, was at least to the base of the roots and in some cases to the subcrown internode. The greatest distance they extended upward was approximately 6 inches. Apparently the fungus advanced upward more readily than downward, which may possibly be attributed to the better aeration near the surface of the soil.

Plants with the inoculum 3 inches or less below the seed became yellow, and stunting was apparent. There was scarcely any indication of yellowing or stunting where the inoculum was 4 or 5 inches below the seed.

Observations indicated that on plants with the inoculum 2 or more inches below the seed the crown became diseased through the secondary roots and subcrown internodes, and that the greater the number of diseased secondary roots that extended to the crown the more severely the crown was diseased. If only one secondary root was diseased from the point of infection to the crown, the crown was diseased only where that diseased root connected with it. In other cases it was evident that the only avenue of entrance to the crown was through the subcrown internode. The general appearance of the plants is shown in figure 5.

During the progress of this work, two types of lesions were present. One was continuous from the position of the inoculum; the other was intermittent, that is, several small isolated lesions were scattered along the root. Evidently the isolated lesions originated from mycelium growing through the soil or from strands growing along the surface of the roots.

INFECTION EXPERIMENTS IN THE FIELD

During 1925–26, an experiment was conducted in the field at Manhattan in which artificial cultures of *Ophiobolus graminis* on a barley-and-oats medium were used as inoculum in the manner described for the experiments conducted in the greenhouse. One-inch layers of the artificially infested soil were placed immediately below and in contact with the seed or 2, 3, 4, 5, or 6 inches below the seed.

The soil was inoculated and planting was done on October 1, 1925, and the readings were made on June 4, 1926. The season was warm and dry and the disease was not abundant.

Where the seed was placed on the inoculum, about 15 percent of the plants were killed and those remaining were badly stunted. At the end of the season the stunted plants had nearly recovered and the heads were well filled. On the plants that were killed there were few or no secondary roots and all the primary roots were diseased. Also, the subcrown internodes, leaf sheaths, and crowns were badly affected. On the plants that recovered, most of the primary roots were diseased but the secondary roots were well developed, only a few of those on the lower part of the crowns being infected.

When the inoculum was placed 2 inches below the seed, some wilting and stunting showed on the aboveground portions of the plants, but not so much as when the inoculum was closer to the seed. The secondary roots were well developed and many had escaped infection. The fungus had advanced up some of the primary roots, and through the subcrown internode to the crown or up some of the secondary roots to the crown, and about three-fourths of an inch up the leaf sheaths. The crowns, however, were not badly infected.

When the inoculum was placed 3 inches below the seed, the aboveground parts appeared normal and the secondary roots were well developed. A considerable number of both primary and secondary roots were diseased, but the infection never advanced to the crowns. Neither the crowns nor the subcrown internodes were attacked.

When the inoculum was placed 4 inches below the seed, the plants were affected in the same way as when the inoculum was placed 3 inches below, except that the lesions were lower on the roots.

When the inoculum was placed 5 or 6 inches below the seed, the aboveground parts showed no effects and the roots were only slightly attacked.

SUMMARY

Ophiobolus graminis was found to occur all through the upper layers and down to a depth of at least 15 inches in naturally infested soil near Manhattan, Kans.

By means of layers of inoculum consisting of naturally infested soil or artificial cultures, placed at different levels in noninfested soil, it was found that serious injury to wheat plants resulted only when the fungus was placed 3 inches or less from the seed.

Injury resulted from abundant invasion of the crowns, the fungus reaching the crowns through the primary roots and subcrown internodes or through the secondary roots or both. When only a few roots were attacked, the others continued to function and additional secondary roots were formed. In such cases the wheat plants attacked survived and yielded fairly well. When numerous roots were attacked and the crown became so abundantly invaded that additional secondary roots could not be formed, the plants died. There were various intergradations between these extremes.

The fungus advanced in the roots farther upward than downward.

PYTHIUM SCLEROTEICHUM N. SP. CAUSING MOTTLE NECROSIS OF SWEETPOTATOES[1]

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INTRODUCTION

In an extended account of mottle necrosis, a distinctive decay affecting the enlarged edible roots of the sweetpotato (*Ipomoea batatas* (L.) Lam.), Harter and Whitney (*9*)[2] in 1927 referred to one of its causal parasites as *Pythium scleroteichum* Drechs., stating in explanation that the fungus represented a new species which the present writer would subsequently describe. The promised description has been long delayed in the hope that the fungus might be induced to exhibit a zoosporangial stage in addition to the sexual stage then known, and thus make possible a statement, at least tolerably complete, with respect to the several phases of main importance in the morphology of oomycetes generally. This hope has as yet not been fulfilled, in spite of the considerable variety of substrata and cultural conditions that have been brought into play. Although past experience with some similarly refractory congeneric forms gives reason to expect that zoospore production may ultimately be induced, the degree of economic importance attaching to the parasite, and consequently the increased desirability of avoiding nomenclatorial complications in connection with it, argues against further delay in providing the nomen nudum with such a description as present knowledge permits. Fortunately the binomial in question has not been applied to any other form in the meantime; nor, on the other hand, has the fungus been described as new under some other name. Indeed, so far as can be determined, the original mycological and phytopathological literature of the intervening period contains no reference to the binomial, and makes no mention either of the fungus or of the disease caused by it such as might imply a first-hand examination. This absence of pertinent references, in spite of the unusually brisk descriptive activity of these years, provides some testimony relevant to the occurrence of *Pythium scleroteichum* in nature.

OCCURRENCE OF PYTHIUM SCLEROTEICHUM IN NATURE

Unlike most of its better known congeners, especially those commonly associated with damping-off of seedlings and rootlet decay of crop plants at later stages of development, the fungus does not appear frequently in isolation plate cultures made from miscellaneous collections of diseased or decaying vegetable materials. Although the writer has examined thousands of cultures prepared in the course of more than a decade from materials representing host plants of numerous species, *Pythium scleroteichum* has been recognized only

[1] Received for publication Aug. 11, 1934; issued January 1935.
[2] Reference is made by number (italic) to Literature Cited, p. 889.

among cultures obtained from sweetpotatoes, and of these more particularly among cultures derived from diseased tissues of the enlarged edible roots affected with mottle necrosis, or from rootlets directly attached to such roots. That its presence in rootlets thus attached ensues often from infection directly from the soil is suggested by positional relationships to the extensive lesions within the massive root. Probably, however, this infection does not usually occur until rather late in the growing season, as the parasite was found only rarely among the various species of *Pythium* comprising an assortment of several hundred cultures isolated from affected rootlets of young sweetpotato plants taken from the hotbed or seed bed at planting time in 1924 in connection with investigations reported by Harter (*7*).

The edible root of the sweetpotato may thus be regarded as the characteristic habitat of *Pythium scleroteichum;* yet it must be emphasized that the same habitat and pathological expression are shared certainly by the congeneric *P. ultimum* Trow, and inferentially, on the evidence of pure cultures isolated from diseased roots in the autumn of 1924, also by an unidentified species of *Phytophthora.* As might be expected, the relative proportions in which these three fungi are obtained from diseased specimens collected in the field vary in different seasons. In 1924, when mottle necrosis was evidently more widely prevalent than usual in Maryland, Delaware, and Virginia, *Pythium ultimum* predominated strongly, accounting for approximately 8 out of every 10 diseased specimens collected at the Arlington Experiment Farm, Rosslyn, Va. The remaining specimens yielded *P. scleroteichum* and the *Phytophthora* species in about equal numbers. However, in the autumn of 1928, when mottle necrosis occurred much less frequently, all of the relatively few diseased specimens found at the Arlington farm yielded cultures of *P. scleroteichum.* Similarly, a half dozen diseased specimens from Conesville, Iowa, in September 1932, and a like number from Indiana in October 1932, all yielded cultures of *P. scleroteichum* without any evidence of mixture with either of the other two fungi.

The identification of material from Iowa and Indiana extended the known distribution of *Pythium scleroteichum* into the Middle Western States and suggested that it and mottle necrosis will ultimately be discovered in other regions where sweetpotatoes are grown. A brief note entitled "New Zealand Sweet Potatoes Diseased" and published presumably by W. G. Smith (*10*) as early as 1884 supplements the historical account given by Harter and Whitney and may be pertinent in connection with the distribution of *P. scleroteichum.* It recorded the receipt in England of "tubers" of *Ipomoea chrysorhiza* Hook., which on being cut open revealed in section numerous brown places similar to those in potato tubers invaded by the late-blight fungus, *Phytophthora infestans* (Mont.) de Bary. On microscopic examination a profuse growth of mycelium together with a few oospores bearing considerable resemblance to those of the genus *Peronospora* were seen, yet the fungus was held to be *Pythium debaryanum* Hesse. As *P. ultimum* has probably more frequently than any other species been designated by Hesse's binomial, this identification would seem to be nomenclatorially intolerable, though perhaps accidental, consonance with findings concerning mottle necrosis. It is altogether unlikely, especially since species of *Pythium* as found in their natural hosts are usually in a condition quite unsatisfactory for the

recognition of many significant morphological features, that Smith's specimens could have supplied the information necessary to distinguish between *P. ultimum* and *P. scleroteichum* even if the respective specific characteristics of these two forms had then been known.

Several peculiarities, particularly the strange labyrinthine course of infection and the pronounced disintegration of host tissue and parasite alike throughout the larger lesions, except in the newly invaded marginal parts of freshly water-soaked appearance, contribute toward making mottle necrosis one of the most remarkable of diseases. As these peculiarities obtain, regardless of which of the several causal parasites happens to be involved, it may be concluded that they are due less to the specific characteristics of the fungi concerned than to the properties of the host tissues affected. The irregularity of the lesions would seem in some way to be related to the anatomical differentiation of tissues within the enlarged root, which tissues in general are, moreover, firmer and much less succulent than those of nearly any other relatively mature massive plant structure now known to be invaded by members of the genus *Pythium*. A somewhat similar though less pronounced irregularity is evident in the edible root of the radish (*Raphanus sativus* L.) affected with "black root", caused by another phycomycetous parasite, *Aphanomyces raphani* Kendrick.

As in naturally infected specimens the final conversion of invaded parts into grayish, somewhat felty, dry masses entails the disintegration of the parasite as well as of the host tissue, attempts to isolate *Pythium scleroteichum* or *P. ultimum* from the central portions of the larger morbid pockets are rarely successful. It is not surprising, therefore, that adherence to customary procedure, in failing to reveal the causal fungi, led to an earlier interpretation of mottle necrosis as a physiological trouble (*8*). Yet isolation of either of the parasites is readily accomplished by employing the technic set forth in another paper (*5*), provided care is taken to start with material excised from the newly invaded water-soaked periphery of actively developing lesions.

CULTURAL CHARACTERISTICS

In pure culture *Pythium scleroteichum* is not very striking to the naked eye, though distinguishable from some congeneric forms frequently encountered as plant parasites. On transparent media of moderate richness like maize-meal decoction agar, its mycelium is generally diffuse without marked luster and without either a pronounced radial or cumulous appearance. Aerial growth on such media is ordinarily absent, though on richer substrata and especially in tube cultures rather meager aerial development may sometimes be observed. With respect to rate of mycelial extension the fungus is to be reckoned among the many members of the genus that are intermediate between the species of unusually rapid growth like *P. butleri* Subr. and *P. ultimum* near the one extreme and the relatively slow-growing forms like *P. complens* Fischer and *P. euthyhyphon* Sideris near the other.

Nor does the vegetative thallus of *Pythium scleroteichum* present any conspicuous features under the microscope, even if the somewhat haphazard manner of branching results in a random disposition of the mycelium and makes its appearance considerably different from that, for example, of *P. debaryanum* or of *P. anandrum* Drechsl., in either of

which radially oriented, straight-forward, axial hyphae bear at fairly regular intervals freely ramifying branches of limited length and characteristically irregular course. The mycelial filaments, though in general somewhat smaller in diameter than those of the coarser members of the genus like *P. butleri*, *P. ultimum*, or *P. anandrum*, certainly do not give the impression of minuteness conveyed by such delicate congeners as *P. complens*. Appressoria are produced in relatively small numbers where the mycelium encounters the surface of a solid body like the base of a Petri dish, occurring as in most congeneric forms as swollen terminal modifications that after functional frustration may grow out into processes of connected sickle-shaped parts.

Pythium scleroteichum appears more exacting in its cultural requirements for the normal development of sexual apparatus than most of the better known congeneric forms associated with plant diseases. Familiar congeneric plant pathogenes like *P. debaryanum*, *P. ultimum*, and *P. butleri* show such satisfactory mycelial growth and form sex organs and normal oospores in such adequate numbers on maize-meal agar made by adding agar-agar to a filtered or strained decoction of maize meal that this easily prepared and readily manipulated medium has come into wide use for the cultivation of species of *Pythium*, *Phytophthora*, *Aphanomyces*, and other phycomycetous genera. On this maize-meal agar medium the fungus under consideration likewise shows good mycelial growth and gives rise to an abundance of oogonia with accompanying antheridia, but degeneration of the oogonial contents is usually so prevalent that only one normal oospore may result from several hundred female structures. If, however, the medium is modified by incorporating in it together with the decoction a considerable quantity of the finer maize-meal sediment and then adding enough hydrochloric acid to change the pH value, ordinarily about 6.0, to 5.0 or 4.5, oogonial degeneration becomes negligible and normal oospores are produced in extraordinary quantity. Similar improvement in production of normal oospores by cultivation on the more substantial maize-meal agar medium has been observed in other members of the genus, notably in the fungus described by Braun (*2*) as *P. complectens* (which, however, would appear to be the same as that on which de Bary based at least one of his figures [*1*, *pl. 5*, *fig. 4*] of his *P. vexans*), in the flax parasite identified by Buisman (*3*) as *Pythium megalacanthum* de Bary, and in the species described by the writer (*6*) as *P. polymastum*, *P. acanthicum*, *P. periplocum*, and *P. myriotylum*.

MORPHOLOGY

The use of a medium suitable for the production of normal sexual structures is especially important because the distinctiveness of *Pythium scleroteichum* attaches very largely to the morphology and relationships of oogonium, antheridium, and oospore. The antheridia vary in number from 1 to 5 and are borne on rather slender longish branches all of which usually (fig. 1, *A–C*, *E–I*; fig. 2, *A–F*, *H*, *I*), though not always (fig. 1, *D*; fig. 2, *G*) represent ramifications of a single parent filament, which may be a branch of the oogonial hypha (fig. 1, *C*, *G*; fig. 2, *F*, *I*), or have some other close connection with the oogonial hypha (fig. 2, *C*, *D*, *H*), or, again, may be merely a neighboring filament without demonstrable connection with the oogonium (fig. 1, *B*, *E*, *F*, *H*, *I*; fig. 2, *A*, *E*). In any case, when the fungus is

cultivated on agar media the hyphal elements directly bearing the male organs are wrapped intimately and extensively about the oogonium in a manner prevalent among various species of *Aphanomyces* parasitic on higher plants (*5*). Similar enwrapment of the oogonium has been noted previously (*6*) in a discussion of *P. periilum* Drechsl., which species would seem to articulate more closely than any other with the one under consideration. In *P. myriotylum* intimate application of the antheridial branches is varied with looser arrangement of these elements, and the same condition obtains, even in larger measure, in *P. graminicolum* Subr. and *P. arrhenomanes* Drechsl. Butler (*4*) found extensive enwrapment of antheridial branches about the oogonia of a fungus parasitic on tobacco in Nyasaland, provisionally designated by him as *P. aphanidermatum* (Eds.) Fitzp. Such disposition of antheridial branches, as Butler intimated, is quite foreign to *P. aphanidermatum* and to the larger *P. butleri*, so that final reference of the tobacco parasite to either of these species would seem definitely precluded. Agreement in sizes of oogonium and of oospore suggests the possibility of identity with *P. scleroteichum*, though the abundant production of zoospores provides a feature strongly at variance with the performance of the latter. In spite of some evident discrepancies, the likelihood of the Nyasaland fungus being *P. myriotylum* deserves consideration, especially as a number of cultures isolated from tobacco seedlings by S. C. J. Jochems in Sumatra and sent to the writer in 1929 were readily identified with that parasite of mostly tropical and subtropical distribution.

In emphasizing the intimate enwrapment of the oogonium by the antheridial branches as a specific characteristic of *Pythium scleroteichum*, it must be mentioned that when the fungus is cultivated under aquatic conditions, the apparatus sometimes is formed away from solid material and this enwrapment then is largely supplanted by a much looser relationship. Apparently the rigidity of a solid, or at least firm, medium in preventing the yielding of one sexual element relative to the other, is of consequence in bringing about the intimate arrangement. However, even if this arrangement were dependent entirely on the merely mechanical properties inhering in a solid substratum, it yet would constitute a feature of importance, since in nature the fungus undoubtedly develops its sex apparatus in such substratum, and even under artificial cultivation has hitherto failed to produce normal oospores in the absence of solid material.

Often the antheridial branches of *Pythium scleroteichum* show a number of sharp transverse indentations or furrows (fig. 1, *E;* fig. 2, *C, D, F, H*) the like of which has never been observed in other members of the genus. The male organ itself, just as in *Pythium periilum*, is generally upcurved from the oogonium, not only being applied distally with its somewhat flattened apex, but often, though not always, also making contact proximately by means of its narrowed basal part. As the wall of the antheridium is appreciably thinner than in some other species like *P. butleri* and *P. ultimum*, this structure, which is besides rather small in comparison with the oogonium, has a characteristically frail appearance. After becoming emptied of contents both the antheridia and the branches bearing them become exceptionally difficult to follow, so that in older cultures only the morphology of the mature oospore and of the surrounding oogonial envelop is clearly revealed.

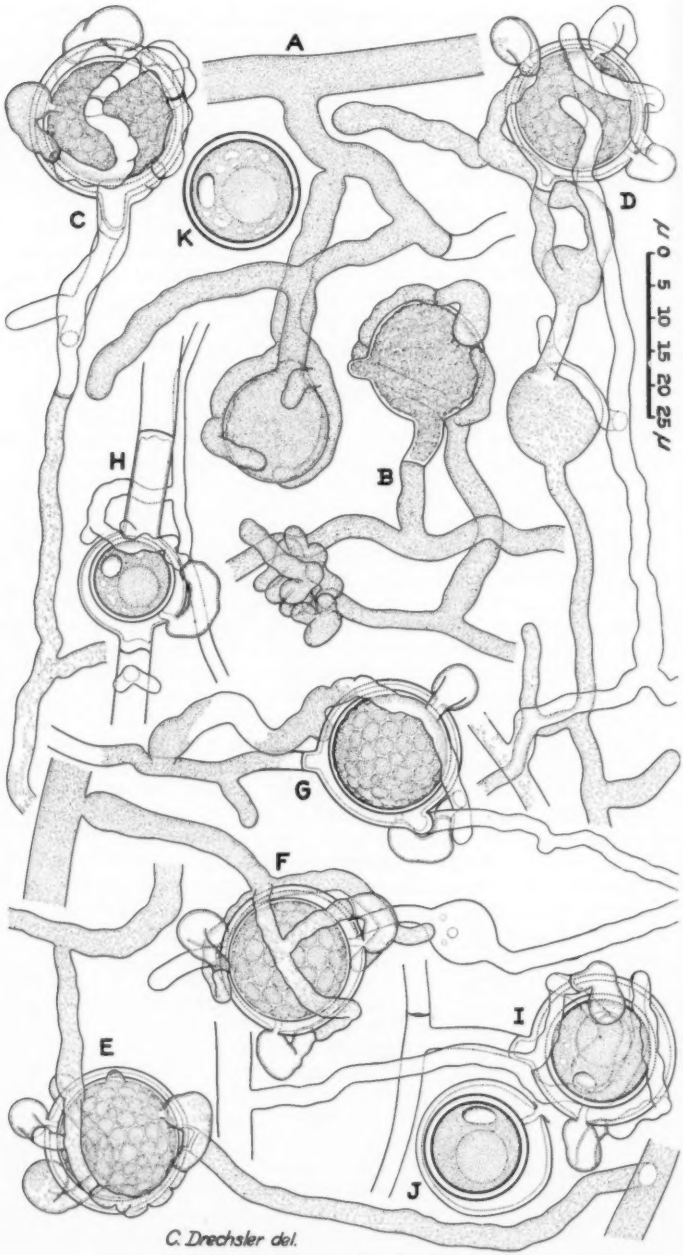

FIGURE 1.—For explanatory legend see opposite page.

With respect to size, neither the oogonium nor the oospore of *Pythium scleroteichum* is exceptional, the diameter in both cases showing a range of magnitudes shared by the corresponding dimensions in a number of better known congeners. Thus 250 oogonia chosen at random in plate cultures of acidulated agar-containing decoction and ample sediment of maize meal, 15 to 20 days after planting, yielded measurements of diameters distributed according to the nearest micron as follows: 16μ, 1; 17μ, 1; 19μ, 1; 20μ, 4; 21μ, 21; 22μ, 22; 23μ, 52; 24μ, 64; 25μ, 43; 26μ, 23; 27μ, 13; 28μ, 3; 30μ, 1; 32μ, 1. Measurements of the diameters of the 250 oospores contained within these oogonia yielded values distributed as follows: 11μ, 1; 13μ, 1; 14μ, 1; 15μ, 7; 16μ, 16; 17μ, 33; 18μ, 47; 19μ, 56; 20μ, 44; 21μ, 30; 22μ, 11; 23μ, 2; 26μ, 1. From these measurements averages of 23.8μ and 18.7μ for diameter of oogonium and of oospore, respectively, were computed, which are not far from the homologous values for *P. ultimum*, *P. paroecandrum*, and *P. aphanidermatum*.

The oospore of *Pythium scleroteichum* is somewhat unusual in being distinctly yellowish when mature, the coloration even in entirely pure culture being comparable in intensity with that usually observed in other species only in isolation plate cultures, that is, in the presence of bacteria and disintegrating host materials. Although coloration in oospores of various species of *Pythium* is often associated with relatively thick oospore walls, this association does not obtain in the fungus under consideration, as its oospore wall is rather thin in comparison with the same structure in congeneric forms (fig. 1, *J*, *K*). On the other hand, except in the areas of contact with the apices of antheridia, the oogonial wall is relatively thick and of unusual resistance to external strain (fig. 1, *J*). Because of this sturdiness the agar medium may dry up without bringing about noticeable distortion of the original spherical shape, whereas under similar conditions the oogonial envelope of *P. ultimum* usually collapses markedly, and that of *P. debaryanum* often loses nearly all semblance of its earlier appearance. As the physical character of the oogonial envelope provides a feature conspicuous even under casual microscopic inspection, it was deemed suitable for recognition in the specific term applied to the fungus.

TECHNICAL DESCRIPTION

Pythium scleroteichum, n.sp.

Intramatrical mycelium in favorable transparent medium diffuse, without lustrous or radiating or markedly cumulous appearance, capable of approximately 26 mm radial extension in 24 hours at 24° C.; consisting of hyphae mostly 2.5μ to 7μ in diameter and bearing in small or in moderate number terminal clavate

EXPLANATORY LEGEND FOR FIGURE 1

Sexual apparatus of *Pythium scleroteichum* developed in plate cultures of acidulated maize-meal suspension agar; drawn with the aid of the camera lucida at a uniform magnification. ×1,000.

A.—Early stage previous to appearance of septum delimiting oogonium and before appearance of antheridia on the clasping branches, the latter being of monoclinous origin.

B.—Somewhat later stage; oogonium and one antheridium delimited by septa; diclinous.

C.—A still later stage; one antheridium emptied and the other discharging contents into oogonium; monoclinous.

D.—A stage shortly following fertilization; one antheridium of monoclinous origin, two of diclinous origin.

E, F.—Diclinous apparatus; one of the antheridial branches in *E* showing transverse indentations.

G.—Oogonium intercalary; closely monoclinous relationship.

H, I.—Mature apparatus; diclinous.

J.—Mature oospore within oogonial wall, showing thickness of oospore wall and of oogonial membrane, as well as size and shape of reserve globule, refringent body, and fertilization tube.

K.—A mature oospore.

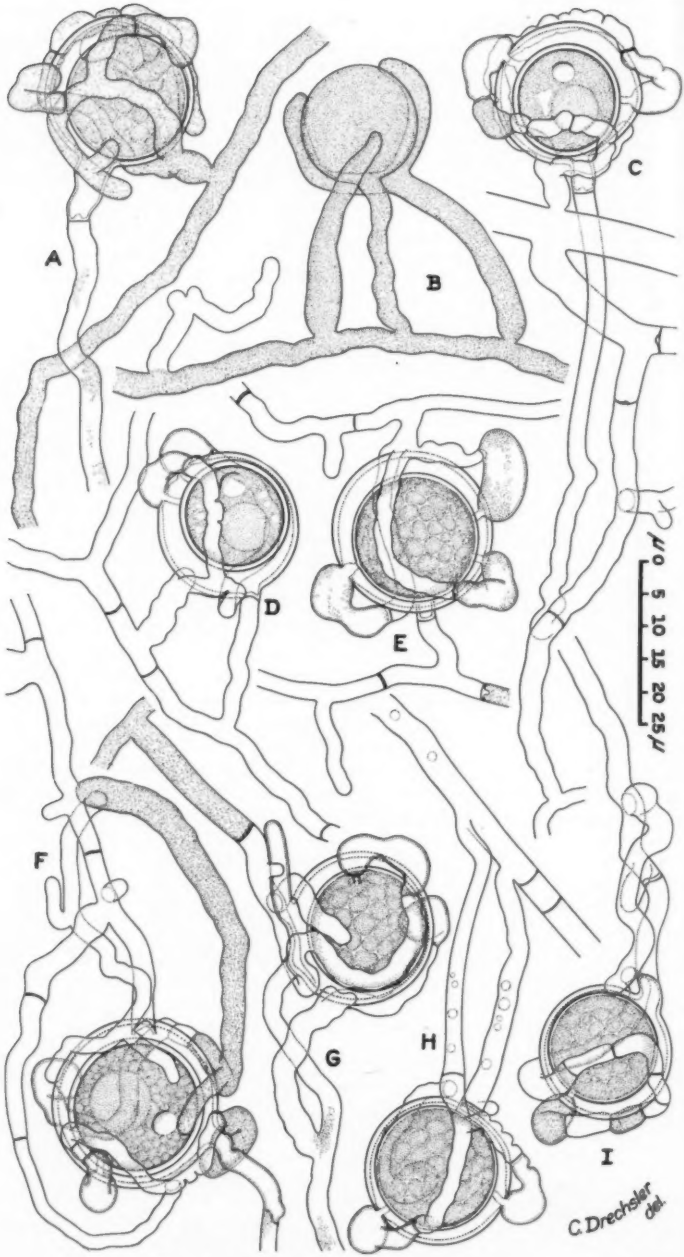

FIGURE 2.—For explanatory legend see opposite page.

appressoria mostly 5μ to 12μ in diameter. Aerial mycelium absent or present in meager quantity.

Oogonium intercalary or more frequently borne terminally on the longer hyphae or on shorter branches; provided with a smooth sturdy wall, 0.5μ to 1.2μ, mostly 0.7μ to 1.1μ in thickness, though conspicuously thinner in areas of contact with apices of antheridia; subspherical, measuring 16μ to 32μ, mostly 21μ to 27μ (average 23.8μ) in diameter, the delimiting septum or septa rather regularly inserted beyond the spherical contour so as to include a cylindrical part or parts, mostly 1μ to 10μ in length. Antheridia regularly clavate, crooknecked, measuring 4μ to 7μ in diameter in wider distal part and 9μ to 16μ in length along curved axis from apex to basal septum, the apical end bluntly rounded and making narrow contact with the oogonium or flattened and more broadly appressed, in either case giving rise to a fertilization tube approximately 1μ in diameter and 1μ to 3μ in length; varying in number usually from 1 to 5 (average 3) to an oogonium; borne usually terminally or occasionally laterally on branches which sometimes contain a number of septa and are often constricted at intervals by abrupt, relatively deep, transverse, dorsal furrows, but which in any case are frequently, together with vegetative ramifications arising from them, wrapped extensively and intimately about the oogonium; antheridial branches supplying an oogonium arising frequently from a single parent hypha, or sometimes from two parent hyphae without close mycelial connection with one another, the single parent element or one of the plural elements often constituting a ramification of the oogonial hypha, the filamentous connection between antheridium and oogonium then not usually measuring less than 75μ in length. Oospore distinctly yellowish, smooth, largely filling oogonium, measuring 11μ to 26μ, mostly 15μ to 22μ (average 18.7μ) in diameter, with a wall 0.8μ to 1.4μ, mostly 0.9μ to 1.3μ, in thickness, a reserve globule 6.5μ to 11.5μ (average about 8.6μ) in diameter, and a refringent body oblate ellipsoidal or subspherical in shape, in latter case mostly 3.5μ to 5μ in diameter.

Causes a mottled decay especially of the enlarged edible root of *Ipomoea batatas* (L.) Lam. in Delaware, Maryland, Virginia, Indiana, and Iowa.

Mycelium ramosum, hyphis 2.5–7μ crassis; oogoniis globosis terminalibus subinde intercalaribus, 16–32μ diam., membrana 0.5–1.2μ crassa; antheridiis 1–5μ, clavulatis, curvulis, 9–16μ longis, 4–7μ crassis; ramis antheridialibus longis, saepius ramosis, ex origine monoclina vel diclina, oogonium amplectentibus; oosporis sphaericis, flavidis, 11–26μ diameter, membrana 0.8–1.4μ crassa; zoosporangiis ignotis.

Hab. parasitice in radicibus Ipomoeae batatae, Delaware, Maryland, Virginia, Ohio, et Iowa.

LITERATURE CITED

(1) BARY, A. DE.
1881. ZUR KENNTNISS DER PERONOSPOREEN. Bot. Ztg. 39: 521–530, 537–544, 553–563, 569–578, 585–595, 601–609, 617–625, illus.

(2) BRAUN, H.
1924. GERANIUM STEMROT CAUSED by PYTHIUM COMPLECTENS N. SP. HOST RESISTANCE REACTIONS: SIGNIFICANCE OF PYTHIUM TYPE OF SPORANGIAL GERMINATION. Jour. Agr. Research 29: 399–419, illus.

(3) BUISMAN, C. J.
1927. ROOT ROTS CAUSED BY PHYCOMYCETES. Meded. Phytopath. Lab. "Willie Commelin Scholten" 11: 1–51, illus.

EXPLANATORY LEGEND FOR FIGURE 2

Sexual apparatus of *Pythium scleroteichum* developed in plate cultures of acidulated maize-meal suspension agar; drawn with the aid of the camera lucida at a uniform magnification. ×1,000.

A.—Stage during fertilization; antheridia of diclinous origin, one lateral and one terminal in position.

B.—An early stage; closely monoclinous.

C.—A mature stage, more remotely monoclinous, one antheridial branch septate, the other showing transverse indentations.

D.—A mature stage, rather closely monoclinous; one of the antheridial branches with transverse indentations.

E.—A stage during fertilization; diclinous.

F.—A mature stage, the oogonium intercalary, the three antheridia of monoclinous origin, but two of them being borne on a bifurcating branch having separate origin from the branch bearing the third.

G.—A stage showing fertilization by two antheridia, one of monoclinous, the other of diclinous origin.

H.—A stage showing three antheridia of monoclinous origin, a branch bearing one of them having transverse indentations.

I.—A stage showing fertilization by a single antheridium borne on a septate branch; monoclinous.

(4) BUTLER, E. J.
1928. REPORT ON SOME DISEASES OF TEA AND TOBACCO IN NYASALAND. 30 pp., illus. Zomba.
(5) DRECHSLER, C.
1929. THE BEET WATER MOLD AND SEVERAL RELATED ROOT PARASITES. Jour. Agr. Research 38: 309–361, illus.
(6) ———
1930. SOME NEW SPECIES OF PYTHIUM. Jour. Wash. Acad. Sci. 20: 398–418.
(7) HARTER, L. L.
1924. PYTHIUM ROOTLET ROT OF SWEET POTATOES. Jour. Agr. Research 29: 53–55, illus.
(8) ——— LAURITZEN, J. I., and WEIMER, J. L.
1923. MOTTLE-NECROSIS OF SWEET POTATOES. (Phytopath. Note) Phytopathology 13: 145–146, illus.
(9) ——— and WHITNEY, W. A.
1927. MOTTLE NECROSIS OF SWEET POTATOES. Jour. Agr. Research 34: 893–914, illus.
(10) S[MITH], W. G.
1884. NEW ZEALAND SWEET POTATOES DISEASED. Gard. Chron. (n.s.) 22: 555.

STORAGE OF COTTON POLLEN [1]

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INTRODUCTION

Investigators who have had experience in hybridizing cotton doubtless have been confronted with the problem of bridging the gaps that often occur between the flowering of plants chosen as pistillate and those chosen as staminate parents. Although the cotton plant normally flowers during a period of several weeks, it does not always flower every day, but may, after several days without flowers, produce several at the same time. In interspecies hybrids especially there is a strong tendency for this condition to become the rule rather than the exception in the second and later generations. When two such hybrid plants have been selected for crossing, it frequently happens that on many days they do not flower simultaneously. This condition may result in failure to obtain sufficient cross-pollinated seed to plant adequate first-generation progenies. Considerable difficulty in this respect has been encountered in the genetic investigations of cotton at the United States Field Station, Sacaton, Ariz.

Storage of pollen appeared to be the solution of the problem. Preliminary tests in 1932 having indicated the feasibility of storing cotton pollen under refrigeration, two experiments were performed in 1933, the results of which are described in this paper.

MATERIAL AND METHODS

The experiments were conducted with Egyptian-type cotton of the Pima variety. All pollinations were made on flowers that had been emasculated before anthesis. An ordinary household refrigerator was used for storing the buds and flowers, and no means were available for controlling the temperature. The minimum temperature recorded in the refrigerator was 40° F. No record of the maximum temperature was kept, but it is a fairly safe assumption that the daily range amounted to 8° to 10°, as the refrigerator was in household use and was situated on a west porch where the only protection from the afternoon sun was a canvas awning. The average temperature was probably not much below that (52°) which Pearson [2] found to give the greatest longevity for pollen of plants of the cabbage group.

The first experiment was conducted during the period August 10 to August 22, inclusive, and the second experiment during the period August 24 to August 31, inclusive. As controls on the results with stored pollen, a given number of flowers were pollinated with fresh pollen on each day of the experiments.

[1] Received for publication Aug. 20, 1934; issued January, 1935.

[2] PEARSON, O. H. BREEDING PLANTS OF THE CABBAGE GROUP. Calif. Agr. Expt. Sta. Bull. 532, 22 pp., illus. 1932.

The sources of pollen were as follows:

Experiment 1

Control, pollen not stored, flowers taken from the plants soon after anthesis had begun.

Flower buds collected late in the afternoon before anthesis, wrapped in waxed paper, and held in the refrigerator for 2, 3, and 4 days.

Flower buds collected late in the afternoon before anthesis and held in the laboratory at air temperature for 2 and 3 days.

Open flowers collected early in the morning, soon after anthesis had begun, wrapped in waxed paper, and placed in the refrigerator for 2, 3, and 4 days.

Open flowers collected at the same time as the preceding and held in the laboratory at air temperature for 2 and 3 days.

Experiment 2

Control, same as in experiment 1.

Loose pollen, from flowers taken from the plants at 1 p. m. on the day of anthesis, shaken into glass trays, covered to prevent absorption of moisture, and held in the refrigerator for 2, 3, and 4 days.

Loose pollen, collected as described above and held in the laboratory in covered glass receptacles at air temperature for 2 and 3 days.

Loose pollen, collected as described above, placed in a desiccator over calcium chloride, and held at air temperature for 2 and 3 days.

The buds and flowers that were stored intact in experiment 1 were enclosed in paper bags as soon as they were taken from the plant, and the bags, in turn, were enclosed in waxed paper. Pollination of the emasculated flowers was effected by brushing the stigmas with the anthers of the stored bud or flower. The loose pollen, in experiment 2, was collected in the afternoon of the day of anthesis, having been shaken from the anthers before storing. When used for pollinating it was applied to the stigmas with a camel's-hair brush.

Figure 1 illustrates the two phases of development of the flowers in which the pollens of experiment 1 were stored. In the case of the refrigerated buds the corolla remained closed after the buds were placed in storage. Opening of the anthers appeared to be complete or nearly so at the time the buds were used for pollinating, but had been delayed approximately 48 hours. In the case of stored buds and flowers the corolla became slightly flacid after 2 days in refrigeration, although it never showed any signs of the discoloration which commonly occurs in cotton flowers in the field a few hours after anthesis.

RESULTS

The results of the experiments are stated in table 1.

It will be noted that in experiment 1 pollen stored in the bud under refrigeration for 2 days was practically as efficient as the fresh (control) pollen, whether the degree of fecundation is measured by the percentage of bolls set or by the mean number of seeds per boll. Thereafter there was a marked decline in the efficiency of the stored pollen, the bolls set having decreased from 88 percent with pollen stored 2 days to 26 percent with pollen stored 4 days, and the mean number of seeds per boll having decreased from 13.4 with pollen stored 2 days to 4.5 with pollen stored 4 days. Pollen stored in the open flower 2 days under refrigeration was also practically as efficient as the fresh (control) pollen, and in this case the decline after further storage was less marked, the percentage of bolls set having been 64

percent and the mean number of seeds per boll 11.6 when the pollen was applied after 4 days of storage. On the other hand, pollen stored at air temperature, either in the bud or in the open flower, lost completely its ability to effect fecundation after 2 days of storage.

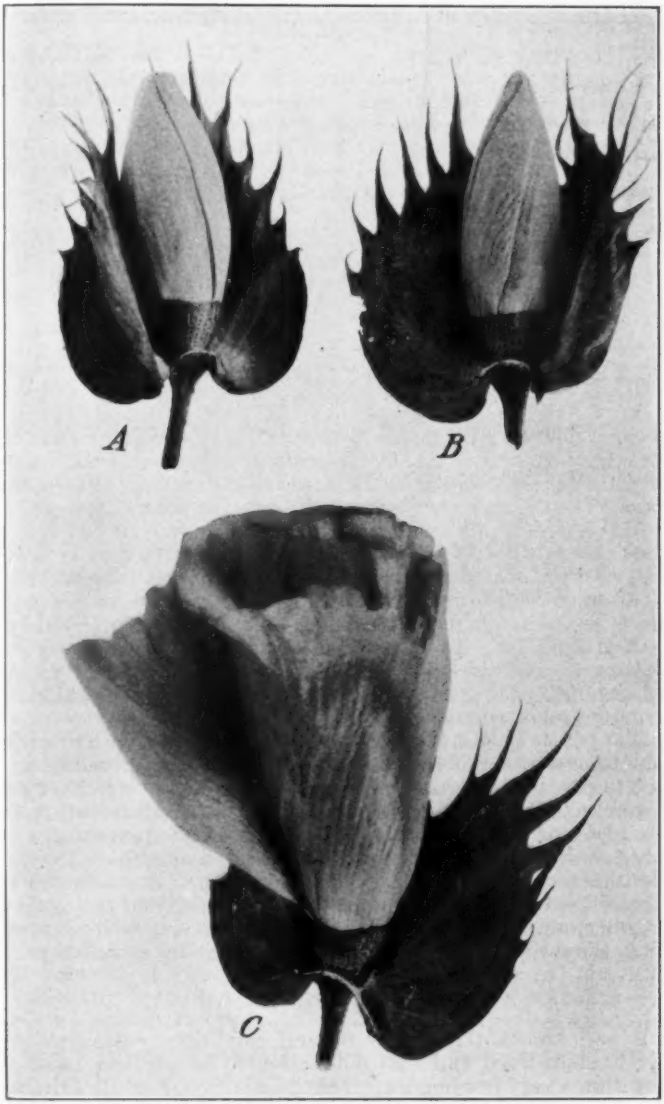

FIGURE 1.—*A* and *B*, Flower buds of Pima Egyptian cotton after being in refrigerator 48 hours; *C*, Pima flower of same age as buds (*A* and *B*) but left in the field to open naturally and placed in refrigerator after its maximum dimensions were obtained. (Photograph by Claude Hope.)

TABLE 1.—*Percentage of bolls set and number of seeds per boll as indications of degree of fecundation effected with fresh (control) pollen and with pollen stored under various conditions*

Condition of storage	Period of storage [1]	Flowers pollinated	Bolls set		Average seeds per boll
	Days	*Number*	*Number*	*Percent*	*Number*
Experiment 1:					
Control	0	50	45	90±2.9	13.5±0.39
Buds, refrigerated	2	50	44	88±3.1	13.4± .44
	3	50	34	68±4.4	11.3± .56
	4	50	13	26±4.2	[2] 4.5± .48
Buds at air temperature	2	50	0		
	3	50	0		
Flowers, refrigerated	2	50	48	96±1.9	13.2± .37
	3	50	41	82±3.7	14.7± .36
	4	50	32	64±4.6	11.6± .59
Flowers at air temperature	2	50	0		
	3	50	0		
Experiment 2:					
Control	0	50	50	100	14.4± .31
Loose pollen, refrigerated	2	51	50	98±1.3	16.0± .30
	3	50	27	54±4.7	8.4± .62
	4	50	8	16±3.5	[2] 5.0± .71
Loose pollen at air temperature	2	50	0		
	3	50	2	4±1.9	2.0
Loose pollen at air temperature and dehydrated	2	50	0		
	3	50	0		

[1] In the case of pollen stored in the bud, in which anthesis was retarded by refrigeration, this does not imply that a corresponding period had elapsed after actual maturity of the pollen.
[2] Probable error increased by Pearson's factor to compensate for small number.

In experiment 2, loose pollen from open flowers, when stored 2 days under refrigeration, was apparently even more efficient than the fresh (control) pollen, the flowers pollinated with it having yielded on an average 1.6 ± 0.45 more seeds per boll than the control flowers. No explanation of this difference is apparent unless it be that in the mechanical operation of collecting the loose pollen the smaller, weaker grains were eliminated by reason of their lightness, whereas in the application of the control pollen by brushing the anthers against the pistils a somewhat higher percentage of inferior pollen grains may have been applied. The degree of fecundation effected by loose pollen stored under refrigeration, whether measured by the percentage of bolls set or by the mean number of seeds per boll, declined rapidly after more prolonged storage.

Similar pollen when stored at air temperature either with or without special provision for dehydration lost completely or nearly so its ability to effect fecundation after 2 days of storage. Of 200 flowers that were pollinated with loose pollen stored at air temperature, only 2 were recorded as having set bolls, and these bolls contained a total of only 4 seeds. It is possible that these seeds resulted from accidental self-fertilization, due to imperfect emasculation. It was noted that the dehydrated pollen grains adhered with difficulty to the surface of the stigmas, although cotton pollen is naturally very well adapted to clinging, the surface of the grains being mucilaginous as well as prickly (fig. 2).

DISCUSSION

It is well known that under natural conditions cotton pollen is relatively short-lived and that pollination to be effective must take place within a very few hours after anthesis. So far as the writers are aware, no attempt has been made to determine the absolute or maxi-

mum longevity of cotton pollen. It should be clearly understood that in this experiment no such attempt was made. According to a summary of all work done on pollen longevity, compiled by Holman and Brubaker [3] in 1926, determinations have been made of the longevity of pollen of 23 families of plants, including 175 genera and 231 species. It appears from the data given by Holman and Brubaker that the only member of the Malvaceae studied previously is *Abutilon darwinii*, the pollen of which survived for 14 days at "air dry" temperature. From the information available it is not clear whether fertilization was actually effected by pollen of this age or whether its viability was determined by culture in an artificial medium.

The data in table 1 indicate that for the purpose of prolonging the viability of cotton pollen the best results are to be expected when pollen is stored at a low temperature in the open flower collected at

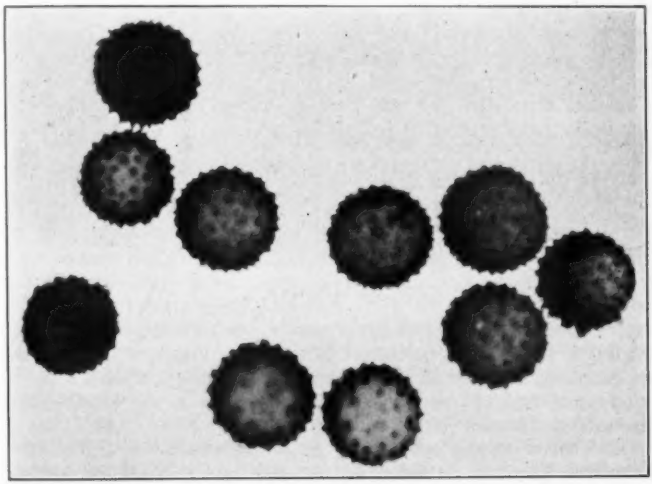

FIGURE 2.—Pollen grains of Pima Egyptian cotton. × 110. (Photomicrograph by Claude Hope.)

the beginning of anthesis. Pollen stored in the loose condition gave the best results on the second day of storage, but after 3 and 4 days of storage, pollen stored in the open flower gave decidedly better results, both in percentage of bolls set and in number of seeds per boll. Under refrigeration, the results were much the same when the pollen was stored in the bud as when it was stored loose.

In a general way these findings conform with those for other stored pollens as reported by Holman and Brubaker.[4] The tendency for the viability of pollen to decline rapidly in storage seems to have been observed by most investigators of the problem, although in the case of many of the longer-lived pollens there is a pronounced tendency for a few of the grains to remain viable long after most of them

[3] HOLMAN, R. M., and BRUBAKER, F. ON THE LONGEVITY OF POLLEN. Calif. Univ. Pubs., Bot. 13: 179-204. 1926.

[4] HOLMAN, R. M., and BRUBAKER, F. See footnote 3.

have lost their vitality. In this connection, Holman and Brubaker state:

> In brief it may be said, then, that in the case of many pollens each lot consists of a relatively large number of grains which retain their viability for a relatively short period and a small number, consisting generally of less than 10 percent of the whole, which survive storage several times as long as the others.

In the case of the refrigerated cotton buds, when used for pollination, the anthers were sometimes just opening on what normally would have been the second day after anthesis. Stored pollen at this stage gave results almost identical with those obtained with the fresh or control pollen and was probably at about the same stage of maturity. Pollen from refrigerated buds, when 3 days old, produced 22 ± 5.3 percent fewer bolls and an average of 2.2 ± 0.68 fewer seeds per boll than did the control. With pollen 4 days old from such buds the difference in favor of the control amounted to 64 ± 5.1 percent of bolls set and 9.0 ± 0.54 mean number of seeds per boll. When it was observed that maturity was delayed approximately 2 days by refrigeration, it was anticipated that the bud stage would prove to be the most effective stage for collecting pollen for storage. The results obtained indicate that delaying the development of the flower does not necessarily result in prolonging the viability of the pollen.

Considering the three methods of storage from the point of view of the labor involved, as well as of their relative efficacy in prolonging viability of the pollen, storage in the flower has marked advantages over storage either in the bud or in the loose condition.

SUMMARY

Cotton pollen was stored successfully under refrigeration, for 2, 3, and 4 days, (1) in buds collected in the afternoon preceding anthesis, (2) in flowers collected in the morning when the anthers were beginning to open, and (3) as loose pollen collected in the afternoon when all the anthers were fully open.

Pollen stored in the laboratory at air temperature failed to effect fertilization, whether collected in the bud in the afternoon preceding anthesis, in the early morning when pollen first became available, or in the afternoon when the pollen was fully mature. Pollen stored in a desiccator at air temperature over calcium chloride also failed to effect fertilization.

With the three methods of storage that proved successful, each additional day of storage resulted in a material reduction in the degree of fecundation effected, whether measured by the percentage of bolls set or by the number of seeds per boll.

No attempt was made to determine the absolute or maximum longevity of cotton pollen.

Storage of pollen by refrigeration in the flower collected early on the day of anthesis appears to be the most practical of the three methods.

CATALASE ACTIVITY IN RELATION TO AGE AND VIABILITY OF SCLEROTIA OF THE COTTON ROOT-ROT FUNGUS[1]

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INTRODUCTION

Since the discovery in 1928 (*6*)[2] of a sclerotium stage in the life history of the cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar, several investigators (*5*, *7*, *8*) have shown that the sclerotial bodies may be found in the soil in almost any part of the infested region. Two methods have been developed to facilitate finding sclerotia in the soil. Where soils are loamy or sandy they may be washed through sieves, sclerotia if present being found on the sieve with the coarse sand particles (*5*). In heavy clay soils the sclerotia generally develop along the cleavage planes, and if pressure is applied to lumps of dry soil they usually break apart along the planes and expose the sclerotia to view (*8*).

It has been found that sclerotia are one of the most important means by which the disease carries over in the soil through winter and unfavorable periods. It is important to determine the age and the vitality of the hold-over bodies so as to have a knowledge of the part they play in maintaining the disease in a particular area.

The sclerotia when first formed are white, but they gradually grow darker with age until they are almost black. The degree of color is of some help in indicating the age, but the writers have found that it is not altogether reliable. Dark-brown sclerotia collected from one field may germinate vigorously, while a lot of similar color from another location may germinate slowly or not at all. These differences in vigor indicate variation in the metabolic condition of the tissue, and since catalase activity in some cases has been shown to parallel the vitality of tissues the catalase determination was suggested as a possible indicator of the age and vigor of the sclerotia.

SIGNIFICANCE OF CATALASE ACTIVITY

A number of investigators have found that catalase activity is useful in indicating the viability of seeds and the maturity of or winter injury to vegetative tissues, and in detecting the first stages of deterioration in shipments of certain fruits. As an indicator of viability in seeds there has not been agreement as to the reliability of the catalase measurement. Shull and Davis (*10*) found that the upper seed of *Xanthium*, which showed delayed germination, failed to increase in catalase activity, as did the lower seed, which germinated promptly. Crocker and Harrington (*2*) found that in seeds of

[1] Received for publication Aug. 20, 1934; issued January, 1935.
[2] Reference is made by number (italic) to Literature Cited, p. 901.

Johnson grass and Sudan grass catalase activity seemed to be an indicator of age rather than of vitality. Davis (*3*) in testing lettuce seed found that catalase activity was not a reliable indicator of viability, although by its determination he was able to distinguish viable seed from dead seed, since catalase became rapidly disorganized in the dead seed when they were soaked in warm water. Carrick (*1*) found a marked decrease in the catalase of apples when a majority of the cells had been killed by freezing.

METHOD OF CATALASE DETERMINATION

The apparatus used for the catalase determinations reported in this paper was somewhat similar to that used by Pope (*9*). It consisted of a pair of leveling burettes (fig. 1, *a*, *a'*) one of which was connected through a three-way stopcock (*b*) to a small wide-mouthed bottle serving as a reaction chamber (*a*). This bottle was closed by a three-hole rubber stopper through which passed a connecting tube (*c*), the stem of a stoppered separatory funnel (*e*), and a by-pass tube (*f*) from the funnel to the reaction chamber.

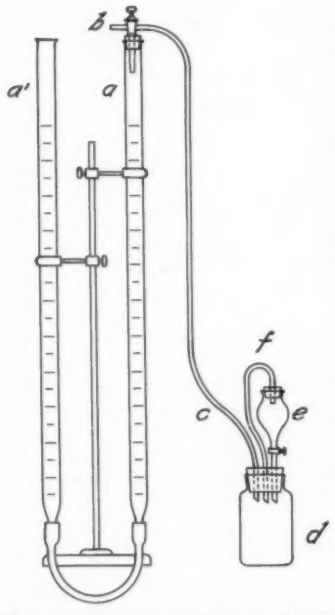

FIGURE 1.—Apparatus used for determination of catalase activity in sclerotia of the cotton root-rot fungus.

The procedure followed in making the tests was essentially the one described by Heinicke (*4*). As a preliminary step a quantity of hydrogen peroxide sufficient for 1 day's tests was rendered neutral to phenolphthalein and checked for oxygen content against manganese dioxide. The container was then placed in a water bath maintained at 25° C. A sample of 8 to 16 sclerotia, weighing about 0.04 g, which after being collected had been stored in deep water, was rapidly dried on blotting paper and weighed on an analytical balance.

Immediately after weighing, the sample was thoroughly macerated in a small agate mortar with about 0.1 g of calcium carbonate, a small quantity of quartz sand, and a little water. After maceration it was washed into the reaction chamber with about 25 ml of distilled water, making a dilution of about 1 to 600. About 3 ml of hydrogen peroxide at 25° was placed in the separatory funnel. The reaction chamber with valve opened to outside air was then attached to the gas-collecting system by the connecting tube and was placed in a water bath to bring its contents to a temperature of 25°. The burettes were leveled with the stopcock open to the outside atmosphere, and the height of the water was recorded. When the reaction chamber and the enclosed sample reached the proper temperature, the stopcock

was turned to connect the reaction chamber to the burettes and to close the opening to the outside. The hydrogen peroxide was admitted to the chamber from the funnel. The chamber was gently agitated in the water bath for 3 minutes, after which the stopcock was turned to close the burette and to open the chamber to the air again. Again the burettes were leveled and read. The difference in the levels before and after the reaction represents the volume of oxygen evolved, which in turn measures the catalase activity.

RESULTS

The material tested was derived both from cultures and from the field. Data relative to the material and the determinations are presented in table 1. The first three lots of sclerotia were removed from a single culture jar 9½ weeks after inoculation. The sclerotia were 1 to 8 weeks old and were separated according to color, which is known to be a rough indicator of age in culture material (*6*). The fourth lot was taken from a culture tube 4 years old in which the sclerotia had been formed about 3 years and 10 months previously. These sclerotia were partly decayed and may have included some bacterial material which affected the catalase activity.

The last 11 lots included in table 1 were obtained from alfalfa fields near Coolidge, Ariz., from infested areas near Sacaton, Ariz., and from a cotton field near Goodyear, Ariz. The ages of the various lots were estimated on the basis of the writers' knowledge of the period when the plants were killed at the point of sampling. It is recognized that this cannot be considered an accurate estimate, since the fungus may remain active long after the host plant has died, and may continue to produce sclerotial tissues by the enlargement of the strands and by forming accretions on the periphery of old sclerotial clusters after long periods of dormancy (*7*). However, it is known that the great majority of the sclerotia are formed during the period in which the fungus is most active in absorbing host tissues. The data for both the pure culture and field samples of sclerotia are arranged in table 1 according to the degree of catalase activity. In the culture material there is no indication that catalase activity is higher in newly formed tissues than in tissues a month or two old. The sclerotia that were nearly 4 years old, however, showed a low catalase content, which in turn paralleled a low percentage of germination.

In the field material there was no indication of differences in catalase according to age or color in the lots from 10 days to 5 months old. In lots older than 5 months, however, there was a sharp reduction in catalase, somewhat in accordance with age and viability. Sample 5, representing field material, is out of line with the other lots in catalase content, showing more than twice the activity of any other sample. It may be that sclerotial tissues, like some seeds, undergo an "after-ripening" shortly after being developed, when the catalase content is at its maximum. The fact that all lots from 5 to 10 weeks old showed unusually high catalase activity lends some support to this theory. The sclerotia in lot 15, which were estimated to be 4 or 5 years old, showed signs of decomposition and were lowest in catalase activity. Twenty-eight percent of the sclerotia in this lot, however, had retained powers of germination. If it is assumed that the estimated ages are approximately correct, there appears to be a fairly

TABLE 1.—*Catalase activity of sclerotia of the cotton root-rot fungus in relation to age, color, and viability*

Source of sclerotia	Group no.	Tests	Germination	Average—						Age of sclerotia (estimated)	Color of sclerotia (Ridgway's standards) [1]
				Weight of sample	Oxygen evolved from sample	Moisture	Dry weight	Oxygen evolved per gram of green tissue	Oxygen evolved per gram of dry matter		
Laboratory culture:		*Number*	*Percent*	*Grams*	*Cc*	*Percent*	*Grams*	*Cc*	*Cc*		
From sand in jar 9½ weeks after inoculation.	1	5	100	0.0436	4.01	68.0	0.0139	92.0	288.5	1 week	Cartridge buff.
	2	4	100	.0451	4.00	69.5	.0138	88.7	289.8	2 to 5 weeks	Cream buff.
	3	3	100	.0343	3.62	66.0	.0117	105.5	309.4	5 to 8 weeks	Hazel.
From 3-foot tube of sand 4 years after inoculation.	4	3	8	.0099	.53	55.0	.0045	53.5	117.8	3 years and 10 months	Black.
Field:											
Alfalfa, recently infested area, Coolidge.	5	5	100	.0487	7.88	78.7	.0104	161.8	759.6	7 to 8 weeks	Buckthorn brown.
	6	4	100	.0418	5.40	58.3	.0174	129.2	310.3	8 to 10 weeks	Sayal brown.
	7	5	100	.0666	6.20	67.3	.0218	93.1	284.4	do	Burnt sienna.
Alfalfa, recently infested area, Sacaton.	8	4	100	.0317	2.95	64.0	.0114	93.1	258.8	10 days	Raw sienna.
	9	4	100	.0665	6.60	59.4	.0270	99.2	244.4	5 months	Hessian brown.
Alfalfa, old infested area, Coolidge.	10	4	100	.0441	3.85	56.0	.0194	87.4	198.5	12 months	Mahogany red.
Alfalfa, recently infested area, Sacaton.	11	2	80	.0285	1.80	64.0	.0103	63.2	174.8	do	Do.
Cotton, old infested area, Goodyear.	12	4	65	.0674	4.40	60.2	.0268	65.3	164.1	Probably 2 years	Very dark Hessian brown.
Alfalfa, recently infested area, Sacaton.	13	2	70	.0291	1.55	66.4	.0080	53.3	158.2	14 months	Dark mahogany.
Alfalfa, old infested area, Coolidge.	14	4	70	.0404	3.20	56.0	.0204	69.0	156.9	12 to 14 months	Dark Hessian brown.
Grass lawn, 4 years after death of elm tree, Sacaton.	15	4	28	.0417	1.90	52.0	.0200	48.0	95.0	4 to 5 years	Blackish brown.[2]

[1] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 pp., illus. Washington, D. C. 1912.
[2] No Ridgway color to match.

close correlation between age and catalase content in the field material. Variations in catalase, however, appear to have no relation to viability until great age is attained, by which time decomposition processes have become apparent.

In collecting the samples, the writers thought that the small sclerotia showed greater decomposition than the larger ones. Consequently, one lot consisting of individuals from the same location was divided into five groups according to size, and catalase determinations were made on each group. Catalase activity was as high for the small sizes in proportion to weight as for the large ones.

Some determinations were made to determine whether drying sclerotial tissues would reduce their catalase activity. It has been shown that rapid drying destroys the vitality of sclerotia (7), and Crocker and Harrington (2) reported that drying depressed the catalase activity in peach and in Johnson grass seeds. Air-drying the sclerotia for 1 hour reduced catalase activity about 50 percent.

SUMMARY

The sclerotia or hold-over bodies of the cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar, darken as they grow older, so that the color of the sclerotia gives some indication of their age. Color is not altogether reliable in this respect, however, nor is it a good indicator of vigor.

As a rule the catalase activity of the macerated tissues of root-rot sclerotia declined with age, but no great or abrupt reduction in activity occurred until the sclerotia began to lose germination power. An unusually high catalase activity in samples 5 to 8 weeks old suggests an "after-ripening" process comparable to that occurring in certain seeds, during which the catalase activity reaches its maximum. Catalase activity seems to parallel closely the metabolic condition of sclerotial tissues and might be used as an indicator of their age and as a test for full vigor, declining vigor, or death.

Catalase activity rapidly decreases when sclerotia are dried.

LITERATURE CITED

(1) Carrick, D. B.
1929. Effect of freezing on the catalase activity of apple fruits. N. Y. (Cornell) Agr. Expt. Sta. Mem. 122, 18 pp.

(2) Crocker, W., and Harrington, G. T.
1918. Catalase and oxidase content of seeds in relation to their dormancy, age, vitality, and respiration. Jour. Agr. Research 15: 137–147, illus.

(3) Davis, W. E.
1926. The use of catalase as a means of determining the viability of seeds. Assoc. Off. Seed Analysts of North Amer. Proc. 18: 33–39, illus.

(4) Heinicke, A. J.
1924. Catalase activity in dormant apple twigs; its relation to the condition of the tissue, respiration, and other factors. N. Y. (Cornell) Agr. Expt. Sta. Mem. 74, 33 pp.

(5) King, C. J., and Hope, C.
1932. Distribution of the cotton root-rot fungus in soil and in plant tissues in relation to control by disinfectants. Jour. Agr. Research 45: 725–740, illus.

(6) ——— and Loomis, H. F.
1929. Further studies of cotton root rot in Arizona, with a description of a sclerotium stage of the fungus. Jour. Agr. Research 39: 641–676, illus.

(7) King, C. J., Loomis, H. F., and Hope, C.
1931. studies on sclerotia and the mycelial strands of the cotton root-rot fungus. Jour. Agr. Research 42: 827–840, illus.
(8) McNamara, H. C., and Hooton, D. R.
1933. sclerotia-forming habits of the cotton root-rot fungus in texas black-land soils. Jour. Agr. Research 46: 807–819, illus.
(9) Pope, M. N.
1932. catalase activity in relation to the growth curve in barley. Jour. Agr. Research 44: 343–355, illus.
(10) Shull, C. A., and Davis, W. B.
1923. delayed germination and catalase activity in xanthium. Bot. Gaz. 75: 268–281.

INFLUENCE OF SMUT INFECTION ON PLANT VIGOR AND OTHER CHARACTERS IN SMUT-RESISTANT OAT VARIETIES[1]

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INTRODUCTION

Results of earlier experiments (*8*)[3] have indicated that covered smut of oats (*Ustilago levis* (Kell. and Sw.) Magn.) may be present as mycelium in the culms of smut-resistant varieties without sporulating in the panicles, and that such latent infection, invisible to the naked eye, may adversely affect the yield of grain per plant, the number of plants, culms, and panicles per row, the height of plants, and perhaps the date of maturity. To test this hypothesis, experiments were started at the United States Northern Great Plains Field Station, Mandan, N. Dak., in 1930 and carried through the 3-year period 1930–32.

REVIEW OF LITERATURE

Not much literature is available on the influence of oat-smut infection on so-called "resistant" varieties. In addition to the results of Stanton et al. (*8*), data are presented in a slightly earlier paper by Bayles and Coffman (*1*) which show a reduction in percentage of seedling emergence and plants maturing from inoculated as compared with noninoculated seed of Markton oats. The significance of this differential, however, is not stressed in the discussion of the data. Gage (*3*), working with susceptible varieties, found that in general slow germination and continued slow development of the oat plant favor sporulation of the pathogenes. Zade (*11*) reports that latent infection of loose smut of oats (*Ustilago avenae* (*Pers.*) *Jens*) reduced the length of culms and panicles and resistance to winter injury. Welsh (*10 p. 163*) reports that probably the most important result of a study of the effect of smut on rust development and plant vigor in susceptible varieties is that "smut infection in oat plants, although not evident in the panicles, may bring about a marked reduction in vigour."

MATERIAL AND METHODS

The oat varieties Black Mesdag, Markton, Navarro, and Victory (C. I.[4] nos. 1766, 2053, 966, and 560, respectively) were used in this experiment. The first three are highly resistant to most physiologic forms of the oat smuts. Victory, highly susceptible to nearly all known forms, was used as the control variety. Other characteristics of these varieties are described in the literature (*2, 7, 8, 9*).

[1] Received for publication Aug. 20, 1934; issued January, 1935.
[2] In cooperation with the Division of Dry Land Agriculture.
[3] Reference is made by number (italic) to Literature Cited, p. 908.
[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases (formerly Office of Cereal Investigations).

Twenty kernels were space-planted 3 inches apart in 5-foot rows 1 foot apart. Two rows of each variety were sown adjacent to each other, one row being sown with smut-free seed and the other with inoculated seed. Five replications (10 rows) of each variety were sown on three dates at 10-day intervals. Thus there were 15 replications or 30 space-planted 5-foot rows of each of the 4 varieties, a total of 120 rows in all each year. The varieties were systematically distributed so that each variety appeared next to each of the others six or more times. The order of seeding was the same in each of the 3 years covered by this study.

The first dates of seeding for 1930, 1931, and 1932 were April 12, 20, and 13, respectively; that is, as early as the ground could be prepared each spring.

Various workers (*1*, *4*, *5*, *6*, *8*, *9*) have shown that there is a large increase in the percentage of infection of loose and covered smuts when the kernels are hulled [5] prior to inoculation and sowing. All seed used in this study was hulled. The seed sown in the even-numbered rows was not inoculated with smut spores. That sown in the odd-numbered rows was blackened with spores of *Ustilago levis* by shaking the hulled seeds with an excess quantity of spores in coin envelopes. The smut spores were representative of the physiologic forms of *U. levis* common in the area. The unsmutted seed was sown first to avoid possible inoculation with smut spores from the inoculated seed.

Notes on height, number of culms, and number of panicles per row were taken in the field immediately after harvest, to avoid any loss of plant parts. As the counts were made the panicles were clipped from the plants, placed in manila harvesting envelopes, and later threshed by a small nursery head thresher and the draft from a small electric fan.

SOIL AND CLIMATIC CONDITIONS

The experiment was conducted on a light, sandy, black loam soil that had been kept in clean fallow during the previous summer.

Climatic conditions were rather adverse to good crop yields, there being a lack of rain in each growing season, and especially in 1930 and 1931. High temperatures and hot winds were of common occurrence each summer. The average precipitation from 1914 to 1932 during the growing season (Apr. 1 to Aug. 31) at Mandan, N. Dak., is 11.22 inches. In 1930 the total precipitation during the growing season was 9.84 inches. In 1931 it was 9.55 inches. In 1932 the total precipitation was 11.40 inches, which is above normal, but the intervals between rains were so long that the plants did not obtain the maximum benefit from each rain.

Minimum and maximum soil temperatures were taken in April and May, 3 inches below the soil surface. These ranged from 35° to 73° F. in 1930 and from 32° to 87° in 1931. The mean temperatures for April and May were 50° and 51°, respectively, in 1930, and 65° and 57°, respectively, for the same months in 1931. The data taken for 1932 were not reliable and are not recorded in this paper.

[5] The term "hulled" is used in this paper to describe seed from which the glumes (hulls) were removed before inoculation with smut spores.

EXPERIMENTAL DATA

Summary data on smut infection, surviving plants, yield of grain, culms and panicles produced, height of plant, and time of heading and ripening for the four varieties are given in table 1.

TABLE 1.—*Influence of smut infection on plant development in resistant oat varieties grown at Mandan, N. Dak., in the 3 years 1930–32*

[Annual average data for each character studied]

Character studied	Susceptible check, Victory		Resistant varieties					
			Black Mesdag		Navarro		Markton	
	Inoculated	Noninoculated	Inoculated	Noninoculated	Inoculated	Noninoculated	Inoculated	Noninoculated
Smut infection:								
Plants per row	5	1	0	0	0	0	0	0
Panicles per row	29	2	1	0	0	0	0	0
Surviving plants:								
Number	132	158	143	158	148	205	139	174
Number per row	9	11	10	11	10	14	9	12
Yield of grain:								
Per row (grams)	24.1	65.9	43.5	51.6	42.6	57.5	66.9	86.0
Per plant (grams)	2.5	5.9	4.1	4.5	4.2	4.2	6.9	7.2
Culms produced:								
Number per row	52	66	52	60	96	129	68	91
Number per plant	6.4	6.4	6.2	6.4	10.6	9.4	8.2	8.4
Panicles produced:								
Number per row	43	58	47	55	75	103	61	83
Number per plant	5.4	5.6	5.6	5.9	8.4	7.5	7.4	7.6
Height of plant (inches)	27	34	34	35	26	27	32	34
First heading	July 10	July 7	July 2	July 2	June 30	June 29	June 30	June 29
Fully ripe	Aug. 7	Aug. 7	Aug. 5	Aug. 5	Aug. 4	Aug. 4	Aug. 6	Aug. 6

SMUT INFECTION

The susceptible Victory developed smutted panicles in 62.5 percent of the plants in the inoculated rows, as compared with 10 percent in the noninoculated rows. Black Mesdag unexpectedly developed an average of two smutted panicles in the inoculated rows in 1932 but was smut-free in 1930 and 1931. No smutted panicles developed in Navarro or Markton during the 3 years.

SURVIVING PLANTS

The smut fungus limited the number of surviving plants in both susceptible and resistant varieties. In Black Mesdag 47.7 percent of the inoculated seed produced mature plants, as compared with 52.7 percent for the noninoculated seed. The corresponding percentages for Navarro were 49.2 and 68.2, and for Markton 46.2 and 57.9, respectively.

YIELD OF GRAIN

Victory, the susceptible check, grown from inoculated seed, produced an average yield of 2.5 g per plant, as compared with an average yield of 5.9 g produced by plants grown from noninoculated seed. The yield per noninoculated row was 2.7 times that of the inoculated. The production of grain in the noninoculated rows of Black Mesdag was 1.2 times that of the inoculated rows, and that of the noninoculated Navarro was 1.3 times that of the inoculated. In 1931 and

1932, average individual plant yields from inoculated rows of Navarro were slightly higher than those from noninoculated rows, owing perhaps to reduced competition because of fewer plants per row in the inoculated rows. The average yield of the noninoculated rows of Markton was 1.3 times that of the inoculated.

NUMBER OF CULMS AND PANICLES PER PLANT

The differences in the number of culms and panicles per plant in the smutted and smut-free rows were small. Victory showed no advantage either way in the number of culms per plant and only a slight advantage in favor of the noninoculated rows in number of panicles per plant. Black Mesdag and Markton show averages only 0.2 of a culm more per plant in the smut-free rows than in the inoculated rows. A similar slight advantage is shown in the number of panicles per plant. Navarro, in contrast to the other varieties, produced 1.2 more culms per plant in the smutted than in the smut-free rows.

HEIGHT OF PLANTS

Plants in the noninoculated rows were taller for each variety than were the plants in the smut-inoculated rows, indicating that smut affected the resistant varieties as well as the susceptible variety. The increased height of plants in the noninoculated rows was easily noticeable in all cases.

HEADING AND RIPENING

There was little difference in the heading and ripening dates of the inoculated and noninoculated rows. Noninoculated rows of Victory averaged 3 days earlier in first heading than the inoculated. In general, smut infection tends to delay the heading dates of both susceptible and resistant sorts. The dates of first heading of Markton and Navarro averaged a day earlier for the noninoculated than for the inoculated rows. The average fully ripe dates for the four varieties were the same for the inoculated and the noninoculated rows, although the average ripening dates for individual years varied slightly for the different varieties. Preharvest weather conditions in the Mandan section commonly tend to bring about premature ripening of grain, lessening variation in dates of maturity.

EARLY, MEDIUM, AND LATE SEEDING

Data on smut infection, number of surviving plants, average yield of grain, etc., for the early, medium, and late seedings, sown 10 days apart, for the 3-year experiment, are of interest. In the susceptible Victory more smutted panicles on an average were produced from early than from medium or late seeding.

For the 3 dates the number of surviving plants varied by an average of 1 to 4 plants per row. The greatest number of surviving plants occurred in the early seedings and the smallest number in the late ones.

For each variety the average yields of the early and medium seedings were greater than those of the late. The average yield of the noninoculated rows was greater for each variety and for each date.

Date of sowing showed no important relation to the average height. The plants from medium and late seedings of Black Mesdag and

Navarro averaged slightly taller in both the inoculated and noninoculated rows than the plants from the early seedings. Victory plants of the inoculated early and medium sowings averaged 1 inch taller than plants from the late. Smut infection for the three dates markedly affected plant height. In every instance the plants grown from inoculated seed were shorter.

Markton plants in the inoculated and noninoculated rows of all dates did not vary in average dates of first heading.

DISCUSSION

The data presented in the foregoing pages show definitely that the resistant varieties, Black Mesdag, Navarro, and Markton, were adversely affected by the smut fungus, although there was no sporulation. This latent infection undoubtedly reduces vigor and productiveness without any external manifestation of smut. In field practice, of course, the resistant varieties are not materially affected, because inoculum is not present in sufficient quantity. Without sporulation inoculum is not produced to infect the next crop.

The average advantage of 1.2 more culms per plant in the inoculated rows of Navarro is difficult to explain. It is probable, however, that the lesser number of plants in the inoculated rows, and hence the lessened competition, were responsible for the increased number of culms per plant. Navarro also naturally produces more culms per plant than Black Mesdag or Markton, which, with the competition factor, may account for the behavior of the inoculated Navarro.

The smaller number of surviving plants suggests greater smut development in plants of the later seedings. More favorable soil temperatures at the time of the late seedings probably account for the rather uniform distribution of surviving plants in the several varieties. The optimum temperature for smut development probably was not reached prior to the late seedings. The plant-mortality rate increased progressively with later date of seeding, which is evidence that smut seriously interferes with germination of the oat seed.

SUMMARY

The smut-resistant varieties Black Mesdag, Markton, and Navarro, and a susceptible variety, Victory, were grown for a 3-year period to test the validity of a hypothesis that covered smut of oats (*Ustilago levis*), present as systemic, latent, or nonsporulating infection, may injuriously affect resistant varieties.

All seed used in this study was hulled. Half of it was inoculated with *Ustilago levis*. Sixty rows of smutted seed and 60 of clean seed, 15 replications of each variety, were sown each year.

The data presented show that smut-resistant varieties grown from inoculated seed were adversely affected even though no smut became visible in the growing plants. The yield per row, the number of plants, panicles, and culms per row, and the height of plants of resistant as well as of susceptible varieties were reduced by smut infection. The reduction of yield was perhaps due chiefly to the reduced number of surviving plants in the inoculated rows and to the presence of nonsporulating infection.

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Smut infection tends to retard the first heading of resistant and susceptible varieties.

Early, medium, and late seedings were made each year. The average yields of the early and medium seedings were greater than those of the late seedings, and in every case the average yield of the noninoculated rows was greater than that of the inoculated rows, indicating perhaps that nonsporulating infection reached its greatest development in the late seedings. The rate of plant mortality increased progressively with the later dates of seeding.

LITERATURE CITED

(1) BAYLES, B. B., and COFFMAN, F. A.
1929. EFFECTS OF DEHULLING SEED AND OF DATE OF SEEDING ON GERMINATION AND SMUT INFECTION IN OATS. Jour. Amer. Soc. Agron. 21: 41–51, illus.

(2) ETHERIDGE, W. C.
1916. A CLASSIFICATION OF THE VARIETIES OF CULTIVATED OATS. N. Y. (Cornell) Agr. Expt. Sta. Mem. 10, pp. 79–172, illus.

(3) GAGE, G. R.
1927. STUDIES OF THE LIFE HISTORY OF USTILAGO AVENAE (PERS.) JENSEN AND OF USTILAGO LEVIS (KELL. & SWING.) MAGN. N. Y. (Cornell) Agr. Expt. Sta. Mem. 109, 35 pp., illus.

(4) JENSEN, J. L.
1888. THE PROPAGATION AND PREVENTION OF SMUT IN OATS AND BARLEY. Jour. Roy. Agr. Soc. England (2) 24: 397–415.

(5) JOHNSTON, C. O.
1927. EFFECTS OF SOIL MOISTURE AND TEMPERATURE AND OF DEHULLING ON THE INFECTION OF OATS BY LOOSE AND COVERED SMUTS. Phytopathology 17: 31–36.

(6) SAMPSON, K.
1929. THE BIOLOGY OF OAT SMUTS. II. VARIETAL RESISTANCE. Ann. Appl. Biol. 16: 65–85, illus.

(7) STANTON, T. R.
1933. NAVARRO OATS. Jour. Amer. Soc. Agron. 25: 108–112, illus.

(8) ——— COFFMAN, F. A., TAPKE, V. F., WIEBE, G. A., SMITH, R. W., and BAYLES, B. B.
1930. INFLUENCE OF HULLING THE CARYOPSIS ON COVERED-SMUT INFECTION AND RELATED PHENOMENA IN OATS. Jour. Agr. Research 41: 621–633.

(9) ——— STEPHENS, D. E., and GAINES, E. F.
1924. MARKTON, AN OAT VARIETY IMMUNE FROM COVERED SMUT. U. S. Dept. Agr. Circ. 324, 8 pp., illus.

(10) WELSH, J. N.
1932. THE EFFECT OF SMUT ON RUST DEVELOPMENT AND PLANT VIGOUR IN OATS. Sci. Agr. 13: 154–164, illus.

(11) ZADE, A.
1932. NEUE UNTERSUCHUNGEN ÜBER DEN LATENTEN PILZEBEFALL UND SEINEN EINFLUSS AUF DIE KULTURPFLANZEN. Fortschr. Landw. 7: 529–532, illus.

METHODS OF OBTAINING EMERGENCE OF TIPHIA ADULTS FROM IMPORTED COCOONS FOR USE AGAINST THE JAPANESE BEETLE[1]

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INTRODUCTION

The successful introduction of foreign parasites belonging to the genus *Tiphia* into the area in the United States infested by the Japanese beetle (*Popillia japonica* Newm.) had come, in 1926, to depend to a considerable extent on the ability to obtain adults from parasites imported in the cocoon stage. Thousands of *Tiphia* were being reared to the cocoon stage abroad and shipped to this country; but, except for a few of the smaller lots, usually of the less desirable species, the emergence was so low that numbers sufficient for effective colonization were not being obtained from the imported cocoons.

Consequently, from 1926 to 1928, an intensive study was made of methods for obtaining increased emergence. A large number of variations of set-up were tried with the nine different species of *Tiphia* which were being imported at that time. In addition, several variations in exposure, in both winter and summer, in ordinary cellar storage, in outdoor soil, and in insectaries, out of soil, were tried. A few of these combinations resulted in partial success with some species; but satisfactory emergence was not obtained from all species until a method was adopted which made use of large incubators in which a high humidity was maintained and temperatures were regulated. In order more easily to regulate temperature and humidity, these incubators were constructed below ground level, and for that reason have been called cellar incubators.

CELLAR INCUBATORS

Although the authors were not successful in obtaining a high emergence, except in a few instances, in tests with plaster-bottom vials, wooden boxes, and other devices in use before the adoption of the cellar incubator, they did gain much helpful information as to the requirements of *Tiphia* cocoons. It was known, of course, that the cocoon stage is always passed in a small subterranean cell, with its attendant slow fluctuations of temperature and continued high humidity. It was found that mortality was almost always extremely high in cocoons that had been torn from their original cells and placed in soil in artificially made cells, or, in fact, whenever they were again subjected to long exposure on a moist earthy substratum. Experience indicated, however, that high humidity and carefully regulated temperatures were essential to good emergence.

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[2] Transferred to the Division of Fruit and Shade Tree Insects, May 16, 1929.
[3] The authors wish to acknowledge the work of J. L. King, in charge of the parasite investigations on the Japanese beetle, and J. K. Holloway, H. A. Jaynes, H. C. Hallock, and J. B. Cronin, all of whom have contributed to the development of the technic now used in rearing *Tiphia*.

Accordingly, cellar incubators were constructed with the idea of reproducing on a large scale the atmospheric environment prevailing in the small earthen cell of a *Tiphia* cocoon. The incubators were made spacious enough to provide both working room for sorting and handling and the space necessary for the storage of large numbers of cocoons from the time they were received until emergence was completed. Each cocoon was stored in a clean, well-ventilated glass vial to free it entirely from contact with damp earthy material and to prevent the rapid spread of fungi from encrusted cocoons to unin-

FIGURE 1.—Interior of a cellar incubator for *Tiphia* cocoons, showing the racks containing trays, the fan for causing air circulation, and the thermocontrol on the wall.

fested ones. Much success in obtaining emergence from *Tiphia* cocoons has been achieved by this method.

By the use of a thermostat the temperature of the cellar incubator (fig. 1) is maintained at approximately the mean temperature of the soil at about the normal depth at which *Tiphia* cocoons are found in their natural habitat. The temperature is changed at 15-day intervals, and the heat is gradually increased from 35° F. in January and February to 76.3° in the latter half of July, and is then decreased again until January. The relative humidity is maintained at approximately 90 percent by evaporation from water which covers the

whole floor of the incubator to the depth of about 1 inch. For the convenience of workers in moving about, "duck boards" are laid on the floor between the stacks. The incubation cellar has two chambers (fig. 2). One is maintained at normal temperatures, and the other,

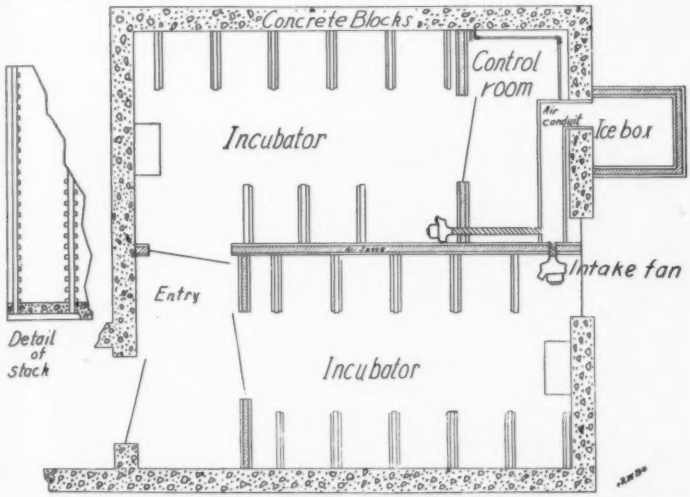


FIGURE 2.—Ground plan of the cellar-incubator unit for obtaining emergence of *Tiphia* from cocoons.

termed the "warm incubator", kept at an average of 5° higher, is used to force those species the emergence of which tends to be unduly retarded at normal temperatures. Trays containing cocoons are stacked so that any one tray can be drawn into the aisle for inspection

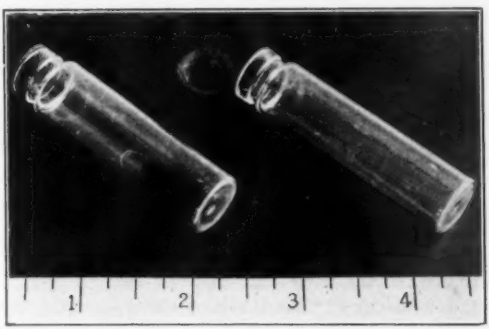


FIGURE 3.—Small homeopathic vials for holding individual *Tiphia* cocoons, and the fine copper-screen stoppers.

without disturbing the others. The cocoons are placed in 2-dram homeopathic vials closed with stoppers of fine copper-wire screen (fig. 3) and are so arranged in trays (fig. 4) as to make frequent inspection convenient. This arrangement of screen-stoppered vials in trays is an adaptation of a similar arrangement used in breeding

parasites at the European corn borer laboratory at Arlington, Mass. Specially constructed vials with necks at both ends have been tried with the idea of obtaining better ventilation for each cocoon.

The incubator is ventilated by drawing in a current of outdoor air by means of a small electric fan. The temperature is controlled, when heating is required, by passing the incoming air over simmering water, and, when cooling is required, by passing it over cracked ice.

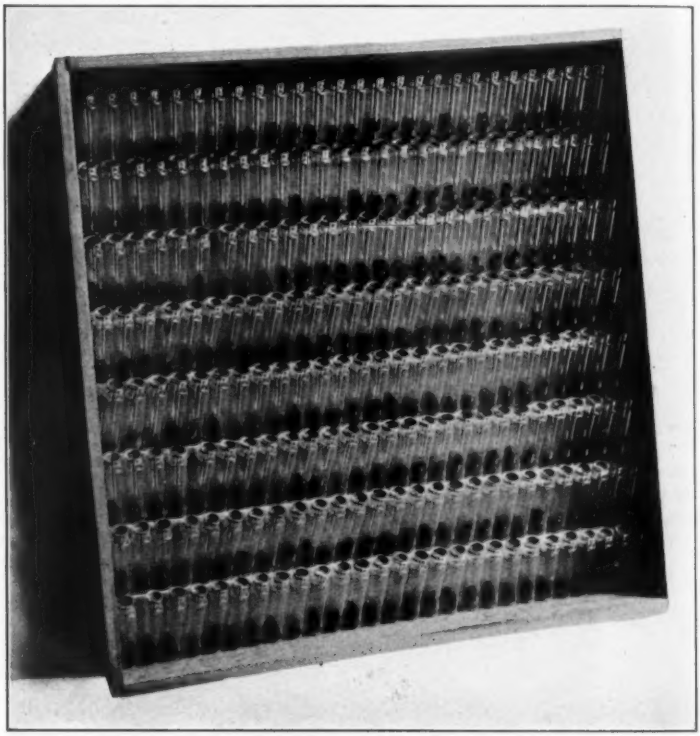


FIGURE 4.—Wooden tray used in the cellar incubator, containing cocoons placed singly in small homeopathic vials.

Ventilation and heating or cooling are simultaneously controlled by connecting the intake fans (fig. 2) with the electrical thermocontrol. Either heating or cooling by this method serves to bring the incoming air to approximately the desired humidity, thus eliminating the danger of excessive desiccation.

Prior to the adoption of the cellar incubator to obtain emergence from *Tiphia* cocoons, several methods of handling, with many variations of construction and exposure, were tried with varying degrees of success.

OTHER METHODS OF HANDLING

PLASTER-BOTTOM VIALS

The use of plaster-bottom vials was one of the earliest methods tried. The vials were made by allowing plaster of paris to harden into a plug about one-half inch thick in the end of a piece of glass tubing 1 inch in diameter and 3 inches long. From 1 to 4 cocoons were placed in each vial. The vials were corked and stacked upright on soil or slightly moistened moss contained in metal pans. The purpose of the plaster was to afford a certain amount of ventilation and to permit the entrance of moisture, which was sprayed over the trays periodically.

WOODEN FLATS

Another method employed was to place cocoons singly in cells about 1 inch square and 2 inches deep, formed by using wooden cross sections to divide greenhouse flats about 18 inches square into about 144 small cells. The cocoons were packed in these cells with moss. The flats were sprayed with water every few days, in an attempt to

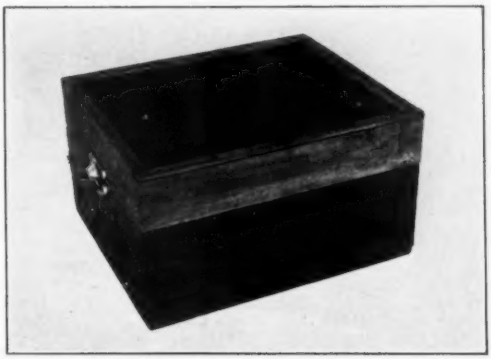


FIGURE 5.—Standard wooden box used for holding soil, and plaster blocks and other set-ups for obtaining emergence of *Tiphia*.

maintain high humidity, and during the summer they were stacked in cloth-walled cages in the insectary.

WOODEN BOXES

The wooden-box method came into use with the adoption of a standard box (fig. 5) for holding cocoons. The box was made of ⅞-inch cypress boards which were resistant to shrinkage, checking, warping, and decay when in contact with the soil. It was of a convenient size—17 inches long, 14 inches wide, and 10 inches deep—for handling when full of soil or other heavy material. Because of its compactness and volume, the box had the advantage, when exposed to the atmospheric conditions of an outdoor insectary, of producing a lag from the wide fluctuations of moisture and temperature in the air surrounding it. The bottom was covered with a fine copper screen, reinforced by a much heavier screen, to provide drainage and aeration and to prevent the escape of parasites or hyperparasites. Interchangeable tops were provided for use at different seasons. Screen-covered tops, similar

to the bottoms, were used on the boxes when they were buried in the soil. Up to the time of emergence the tops of the boxes that were kept in the insectary were covered with black cloth; then these covers were replaced by the so-called "emergence tops" (fig. 6), with trap vials for capturing the issuing adults. The principal methods used for keeping cocoons in these wooden boxes will be described briefly.

According to one method the cocoons in the boxes were placed singly in cell-like impressions made in moist, firm loam. A coarse screen and some loose soil were placed over the cocoons in an attempt to reproduce the earthen cell of the cocoon as it occurs in nature. Another method made use of plaster of paris blocks about 1 inch thick and honeycombed with cells about the size of a normal cell in the soil. Cocoons were packed in these blocks with and without moss. According to another method shallow wooden cross-section trays with cells of about 1 cubic inch capacity were used. In these, cocoons were either placed on or embedded in moist moss, one to each cell. The trays were packed somewhat like brood frames in

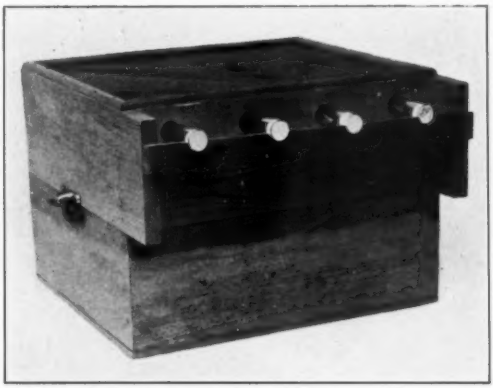


FIGURE 6.—Wooden box capped with an emergence top having trap vials for capturing *Tiphia* adults.

a beehive, with moss between and on top of the frames. Sometimes cocoons were either mixed haphazard in boxes with moist moss or strewn over the surfaces of a tier of small wooden platforms, and then covered with loose moss. Three other methods made use of plaster-bottom vials (as described above or with cloth caps), penny match boxes with holes punched in the ends, and small tins of about ½-inch capacity. These receptacles served as unit containers for single cocoons, and were packed in the wooden boxes. One other method was adapted from a method devised by Prell,[4] in which the cocoons were spread over small frames covered with cloth netting or wire screening, the frames being stacked in the wooden box over a pan of water. This method was quite similar in principle to the method used later for maintaining humidity in the cellar incubators.

The methods in which plaster-bottom vials and wooden flats were used were unsuccessful, apparently because too great fluctuations in humidity and temperature were allowed. The wooden-box methods reduced these fluctuations but not sufficiently, and this factor

[4] PRELL, H. ON A HIBERNATING CAGE FOR INSECTS. Jour. Econ. Ent. 20: 830-834, illus. 1927.

together with others—such as the disinclination of some *Tiphia* adults to leave the packing moss after emergence and the care required to maintain optimum conditions within the boxes—caused these boxes to be considered rather unsatisfactory. With a few of the more hardy species and some variations in arrangement and exposure, however, emergence was satisfactory.

EMERGENCE OBTAINED

Before the cocoons were placed for emergence, a few of each lot were cut open and examined. The numbers examined were, in most instances, too small for determining viability except in a very general way. The viability was notably low in only one lot, of the species *pullivora*. This instance is discussed under the emergence for that species. Emergence totals and percentages are based on the total number of cocoons originally placed for emergence, including those subsequently discarded because of the development of fungus or other evidence of mortality.

The use of cellar incubators had resulted at the end of 1928 in an average emergence from all lots of 35.2 percent (table 1), as compared

TABLE 1.—*Emergence obtained from species of Tiphia in different experimental set-ups*

Species	Plaster-bottom vials in metal pans		Wooden flats		Wooden boxes		Cellar incubators	
	Cocoons	Emergence	Cocoons	Emergence	Cocoons	Emergence	Cocoons	Emergence
	Number	*Percent*	*Number*	*Percent*	*Number*	*Percent*	*Number*	*Percent*
T. pullivora	3,660	0.1			19,325	0.2	22,650	27.5
T. popilliavora, Chinese race			1,216	1.0	10,141	4.1	2,958	87.4
T. popilliavora, Korean race	32	46.9			864	7.4		
T. vernalis			2,548	6.7	8,369	5.6	762	21.2
T. bisecutata					5,173	13.7	1,099	61.1
T. bicarinata					2,542	55.9		
T. asericae					515	35.7		
T. lutopunctata	29	13.8			385	19.5		
T. antigenata							166	42.8
Total	3,721	.6	3,764	4.9	47,314	7.1	27,635	35.2

with an average of 7.1 percent from all arrangements in the standard wooden boxes, and even less from the wooden flats and plaster-bottom vials. The increase resulting from the use of cellar incubators has been twofold or more in all species where data permit direct comparison. In the case of *Tiphia pullivora*, the increase from 0.2 percent for wooden boxes to 27.5 percent for the cellar incubator is more than a hundredfold. The great increase in emergence resulting from the use of cellar incubators is shown graphically in figure 7 for four species of *Tiphia*. In this figure the comparison is made between variations of cellar-incubator treatment and several variations of set-up in wooden boxes, which were the most effective devices used prior to the adoption of cellar incubators.

TABLE 2.—*Total number of cocoons handled each year and the emergence obtained from different species of Tiphia, 1926–28*

Species	1926 [1]			1927 [1]			1928 [2]		
	Cocoons	Adults emerging		Cocoons	Adults emerging		Cocoons	Adults emerging	
	Number	*Number*	*Percent*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
T. pullivora	12,866	13	0.1	27,821	8	0.03	26,352	6,571	24.9
T. popilliavora, Chinese race				8,121	520	6.4	13,122	4,527	34.5
T. popilliavora, Korean race	32	15	46.9	864	64	7.4			
T. vernalis	4,960	270	5.4	5,211	334	6.4	2,380	286	12.0
T. bisecculata	867	325	37.5	1,675	491	29.3	5,256	1,146	21.8
T. bicarinata	720	371	51.5	3,083	1,051	34.1			
T. asericae							799	231	28.9
T. totopunctata	29	4	13.8	583	75	12.8			
T. antigenata							166	71	42.8
Total	19,474	998	5.1	47,358	2,543	5.4	48,075	12,832	26.7

[1] Before the adoption of cellar incubators.
[2] Cellar incubators used for a large portion of all cocoons.

The direct effect of the use of cellar incubators in increasing emergence is shown in table 2. In 1926, before the adoption of cellar

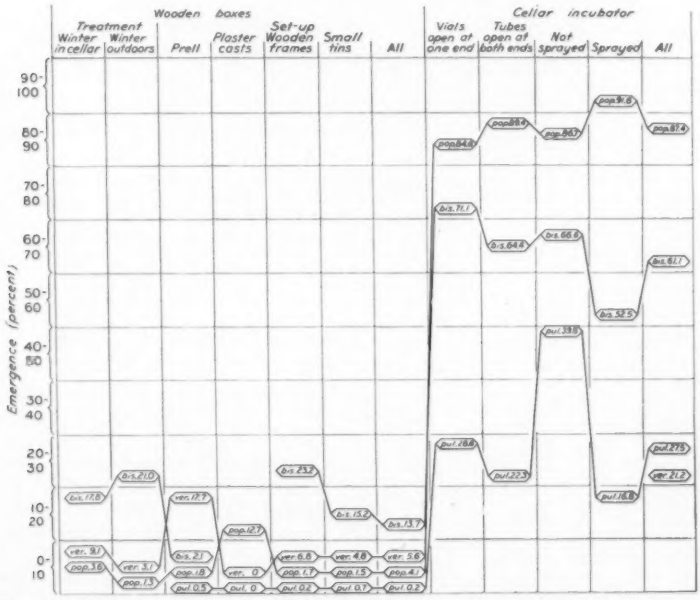


FIGURE 7.—Emergence of four species of *Tiphia* under different arrangements, treatments, and methods: *bis.*, *bisecculata*; *ver.*, *vernalis*; *pop.*, *popilliavora*, Chinese race; and *pul.*, *pullivora*. (The figures in the blocks indicate percentages of emergence.)

incubators, an emergence of 5.1 percent (998 adults) was obtained from 19,474 cocoons. In the following year, also before cellar incubators were used, the emergence increased slightly to 5.4 percent, but

only 2,543 adults were obtained from 47,358 cocoons. In 1928, when cellar incubators were used for a large portion of all the cocoons, an emergence of 26.7 percent was obtained, and 12,832 adults emerged from about the same number of cocoons as were received the previous season. In other words, the number of adult *Tiphia* obtained for colonization from imported cocoons had increased from less than 1,000 per year in 1926 to more than 12,000 in 1928, owing in part to the increase in the number of cocoons received but mainly to the use of cellar incubators.

The reaction of the various species of *Tiphia* to the efforts made to obtain a satisfactory emergence differed in several important respects. The problem of handling cocoons became not only one of obtaining high emergence, but also of obtaining this emergence during the first season following cocoon formation, and restricted as closely as possible to the period during which the stage of development of the host makes the liberation of parasites desirable. This is more fully discussed

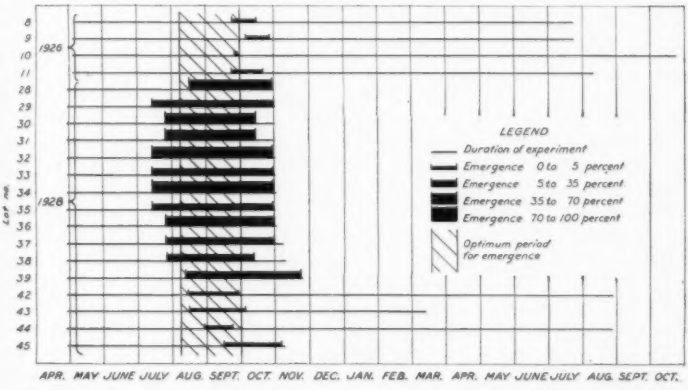


FIGURE 8.—Emergence of *Tiphia pullivora* under various set-ups and conditions of treatment.

under the different species, and under variations in emergence obtained in the cellar incubators.

TIPHIA PULLIVORA A. AND J.

It has been more difficult to obtain a satisfactory emergence with *Tiphia pullivora* than with any other species that has been handled in large numbers. As shown in figure 8, 45 different lots of cocoons (those with no emergence omitted) have been subjected to many variations in treatment. Up to 1928, only 4 lots out of 27 had produced any adult *Tiphia*. With these 27 lots, several variations of method, based on the use of plaster-bottom vials and wooden boxes, were tried. Even in the 4 lots in which emergence was obtained it was less than 5 percent and most of it occurred in October, after the optimum period for liberation of this species was past. Lots 28 to 39, inclusive, were all placed in the incubation cellar, where they were subjected to a number of variations in treatment. The significant fact is that in all of these lots the emergence was fairly satisfactory, in strong contrast with that previously obtained. Furthermore, this emergence occurred during

a period which spanned the optimum period for liberation, without showing the same tendency to hold over to the second season, displayed by lots not placed in the incubation cellar. Lot 45 was the only one placed in the cellar incubators which showed poor emergence, and this was due to the low vitality of the lot when it was received and not to subsequent treatment. Lots 42 to 44, inclusive, were subjected to three variations of the wooden-box method, the same stock being used as for the lots tested in cellar incubators, and serving as a direct control on the latter. As a result of the use of the cellar-incubation method, emergence increased (table 2) from 13 adults and 0.1 percent in 1926, when plaster-bottom vials were used, to 6,571 individuals and 24.9 percent in 1928. In the cellar incubators, the best emergence for any lot was 45.0 percent obtained from lot 36 (fig. 8), in which the vials were open at one end only, were not sprayed with water, and were kept throughout their incubation in the cellar, which was maintained at normal temperature.

TIPHIA POPILLIAVORA ROH., CHINESE RACE

Forty-two different combinations of arrangement and treatment, in which over 21,000 cocoons were used, have been tried with *Tiphia popilliavora* (fig. 9). Up to 1928, 24 separate lots had been set up, and rather interesting results obtained. Only a few lots (omitted in fig. 9) had failed to produce any emergence. An emergence of only 1.0 percent was obtained from cocoons placed in wooden flats (table 1).

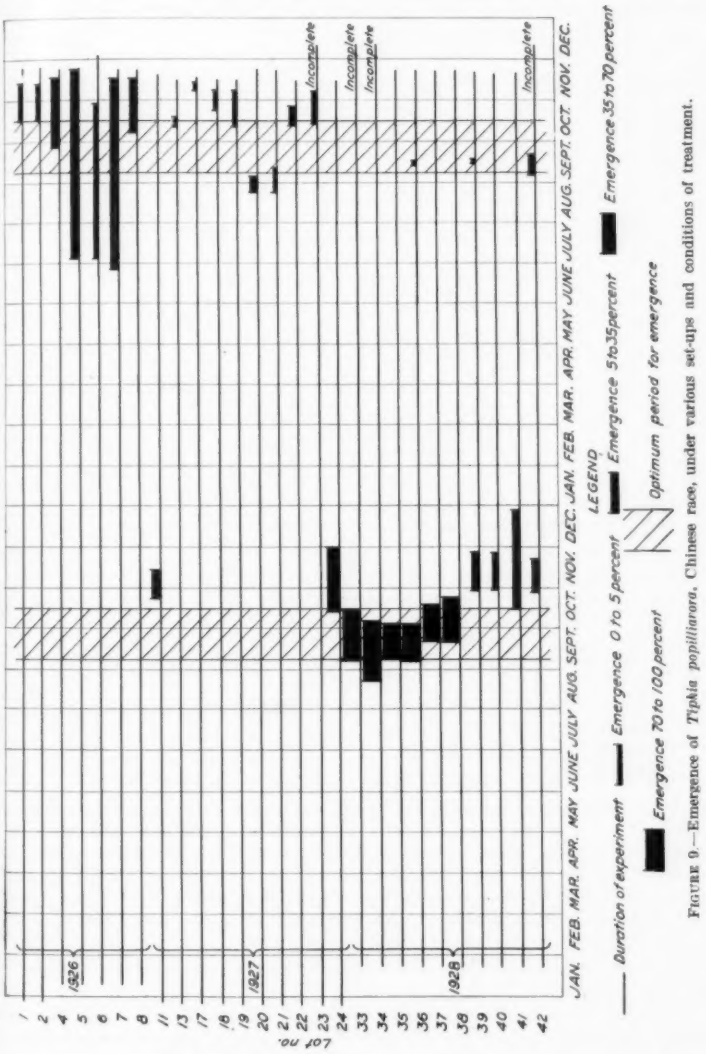
From the several variations of the wooden-box arrangement, an average of only 4.1 percent emergence occurred, although 21.0 percent was obtained from one lot (8, fig. 9) exposed on tiers of wooden platforms, and an average of 12.7 percent from four other lots (4, 5, 6, and 7, fig. 9), placed in cells in plaster blocks. Since the type of exposure of the box arrangements varied widely, the only significant fact for this group as a whole is that under certain favorable combinations of set-up and exposure a fair emergence can be obtained for this strain by the use of wooden boxes. Comparing the emergences obtained from wooden boxes (fig. 9, 1926–27), it is seen that in all instances except two (lots 11 and 24) emergence occurred in the second year. The only instances where emergence from lots maintained in wooden boxes was in excess of 5 percent and also coincided well with the optimum period for liberation were in the case of lots 4, 5, and 7. Even in these instances the overlap into periods not so desirable for liberation was extensive.

Lots 33 to 42, inclusive, represent variations of exposure in cellar incubators. Lots 39 to 42, inclusive, were not brought into the incubators until September and, as a result, the emergence was relatively low and occurred after the optimum period for liberation. In the 6 lots, 33 to 38, which were maintained in the cellar incubators throughout the summer, very satisfactory emergence was obtained, both as to amount and as to synchronization with the optimum period for liberation. The average emergence for these 6 lots was 87.4 percent, and for the single best lot (33) it was 91.6 percent.

Considering the heavy emergence for the lot, the persistence with which living pupae remained in the cocoons even after long exposure in the warm cellar incubator, is difficult to account for, but it was unimportant in the manipulation of the species since it involved only the few cocoons remaining after the first period of heavy emergence.

TIPHIA VERNALIS ROH.

Emergence of *Tiphia vernalis* increased from 5.4 percent in 1926 (table 2), when wooden flats and wooden boxes were used, to 6.4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1926
1927
1928
1 2 4 5 6 7 8 11 13 17 18 19 20 21 22 23 24 33 34 35 36 37 38 39 40 41 42
LEGEND
Duration of experiment
Emergence 0 to 5 percent
Emergence 5 to 35 percent
Emergence 35 to 70 percent
Emergence 70 to 100 percent
Optimum period for emergence

FIGURE 9.—Emergence of *Tiphia popilliavora*, Chinese race, under various set-ups and conditions of treatment.

percent in 1927, when wooden boxes only were used. Some of the modifications of the wooden-box arrangement were highly unsatisfactory, but the modified Prell arrangement resulted in an average emergence of 17.7 percent and was considered fairly satisfactory.

When cellar incubators came into use the average emergence increased to 12.0 percent, which was better than had been obtained with either the wooden boxes or the wooden flats. With all methods of obtaining emergence, its synchronization with the optimum period for liberation in April, May, and June was quite satisfactory.

TIPHIA BISECULATA A. AND J.

A fair percentage of emergence of *Tiphia biseculata* had been obtained in the several variations of the wooden-box set-up before cellar incubators were adopted. The average emergence from all variations of the wooden-box method was 13.7 percent (fig. 7). The average

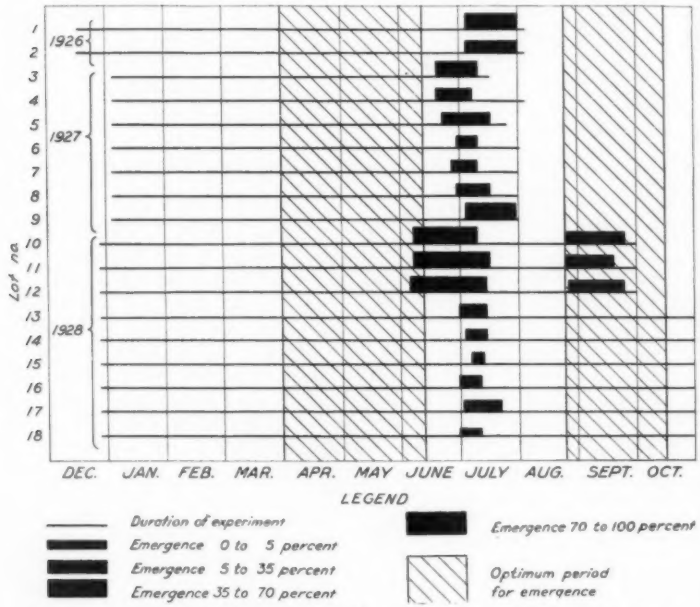

FIGURE 10.—Emergence of *Tiphia biseculata* under various set-ups and conditions of treatment.

emergence in cellar incubators was 61.1 percent, which is considerably better than the best results obtained with wooden boxes. The results obtained from the 18 lots tested are shown in figure 10. An emergence in excess of 5 percent was obtained in each of the 18 lots, except lot 18 which was in a modified Prell wooden-box arrangement. Three variations of the wooden-box method, lots 1, 3, and 9, resulted in emergences greater than 35 percent. The best results, however, were obtained from lots 10, 11, and 12, all of which were subjected to variations of the cellar-incubator method, and which were the only lots in which some form of wooden box was not used.

On the whole, the period of emergence from wooden boxes has been rather short. Two unexpected results from the use of the cellar incubators was the excessive lengthening of the emergence period, and the division of the emergence into two distinct periods separated by a

midsummer interval of nearly 1½ months, in which there was no emergence. These two distinct emergence periods occurred in a stock of cocoons reared from second-generation females collected at Miho, Japan, the preceding season, all of the cocoons having received essentially the same treatment from time of formation until emergence.

Tiphia biseculata has always emerged the first year after cocoon formation, and its emergence has always been poorly synchronized with the prevalence of the proper stage of host grubs of the Japanese beetle, the adult parasites appearing between the spring and late summer periods of abundance of third-instar *Popillia* grubs. This deficiency was partially overcome in the three lots carried in the warm cellar incubator in which the earlier emergence of the first period and all of that of the second period occurred during the optimum periods for adult activity.

OTHER SPECIES

Two other species have been given a preliminary trial in cellar incubators. Trays containing cocoons of *Tiphia asericae* A. and J. were transferred out of the cellar incubator at Moorestown, N. J., to an outdoor insectary at Westbury, N. Y., preceding emergence. From this lot an emergence of 28.9 percent was obtained. Although fairly good, this emergence does not truly represent the effectiveness of cellar incubators for this species, because the cocoons were removed before emergence had taken place. An emergence of 42.8 percent was obtained from a small lot of cocoons of *Tiphia antigenata* maintained in a warm cellar incubator. With *Tiphia bicarinata*, which was not tested in cellar incubators, the emergence was 52.8 and 58.5 percent in two variations of the wooden-box arrangement. With *Tiphia totopunctata*, also not tried in cellar incubators, the emergence was 18.3 and 21.0 percent in two types of wooden-box arrangement, and even in plaster-bottom vials an emergence of 13.8 percent was recorded. For *Tiphia asericae*, a fairly satisfactory emergence (35.7 percent) was obtained with the wooden-box method. With species as hardy as the three last mentioned, it is evident that fair emergence could be obtained without cellar incubators, but the use of the latter, even with species such as these, would undoubtedly greatly increase the amount of emergence of adult *Tiphia* and also help to synchronize this emergence with the period of host-grub infestation.

VARIATIONS IN EMERGENCE OBTAINED IN CELLAR INCUBATORS

Although the use of the cellar-incubator method has consistently resulted in higher emergence from *Tiphia* cocoons than other methods tried, it has been found that variations in treatment within the cellar incubator give noticeably different results. From a study of the reactions of three species (*popilliavora*, *biseculata*, and *pullivora*), it is evident that small differences in methods of handling cause marked variations in the percentages of adults emerging, and that the variation is a matter linked with the individual reaction of each species.

Vials open at one end, referred to as single-end vials, and vials open at both ends, referred to as double-end vials, were available for experimental work. When the double-end vials were used with the species *popilliavora* (fig. 7) the emergence was nearly 5 percent better than when single-end vials were used. However, with two other species, *biseculata* and *pullivora*, the double-end vials gave poorer results than

the single-end vials, the emergence from the double-end vials being 7 percent less for the former species, and 6 percent less for the latter.

Species maintained in the cellar incubators reacted differently to spraying with water (fig. 7). With *popilliavora* the water spray was beneficial and resulted in an increase of nearly 5 percent in the emergence. In the case of *biseculata*, however, the water spray reduced the emergence nearly 14 percent, and in *pullivora* the emergence was reduced 23 percent to a total of only 16.8 percent.

SUMMARY AND CONCLUSIONS

It has been found difficult to obtain emergence from cocoons of most species of *Tiphia* by ordinary insectary methods. By the use of cellar incubators with regulated temperature, humidity, and ventilation, excellent emergence of the hardier species has been obtained, and satisfactory emergence of even the more delicate species. Thus, one of the difficulties in the successful introduction of *Tiphia* from remote regions has been removed. The emergence from cocoons of several species in a number of arrangements tried before the use of cellar incubators was unsatisfactory. From the cocoons of other, hardier species, emergence was fairly satisfactory in several different set-ups and treatments. Emergence from cocoons of all species in the cellar incubators has been so much better than that obtained in any other way that by the end of 1928 this method was being used almost exclusively for *Tiphia* at the Japanese beetle laboratory. Experience indicates the desirability of working out the minor details of treatment for each species separately, as no two species respond identically to all variations of treatment.

NATURAL CROSSING IN THE PIGEONPEA [1]

By C. P. WILSIE, *agronomist*, and MAKOTO TAKAHASHI, *assistant in agronomy*, *Hawaii Agricultural Experiment Station*

INTRODUCTION

The amount of natural crossing in self-fertilized species is of considerable importance, both from the standpoint of maintaining the purity of agricultural varieties and of developing a technic suitable for the isolation of pure lines. Investigators have frequently shown that among certain self-fertilized crops there is enough natural crossing between varieties to interfere markedly with the preservation of genetical purity of desirable agricultural strains, unless by some careful method cross-fertilization is prevented. This is usually accomplished either by growing the desirable strains in complete isolation or by covering the unopened buds to prevent insect visitation to the flowers. Where a large number of varieties are grown together in the breeding nursery, the complete isolation of strains is precluded, and the more laborious and expensive method of covering unopened buds must be resorted to.

The pigeonpea (*Cajanus indicus* Spreng.), a prolific leguminous shrub used extensively in the Tropics for both food and forage, is considered to be normally a self-fertilized species. Many varieties of this legume have been introduced into Hawaii from tropical regions the world over. These varieties have been grown in the breeding nursery, the more promising types being subjected to single-plant selection, hybridization, and progeny testing. The early work covering the experiments conducted in Hawaii on this species has been adequately reported by Krauss (*1*, *2*, *3*).[2] The method used in the isolation of strains was to select desirable single plants in the field, harvest their seed, and grow progeny plots from these selections. This single-plant selection was continued for a number of generations, the object being to establish pure breeding strains desirable in themselves, or to be used in genetical analysis.

When the senior writer took charge of the breeding work at the University of Hawaii in 1931, the nursery plots showed considerable evidence of the results of natural crossing. The following year an experiment was initiated to determine the amount of natural crossing that takes place between varieties in adjacent rows in the nursery or field.

EXPERIMENTAL DATA

The determination of natural crossing was readily made possible by the selection of such characters that the heterozygotes, or natural hybrids, could be readily distinguished in the hybrid generation among the plants of the recessive strain.

Previous work by Krauss (*2*), Shaw (*5*, *6*), and Mahta and Dave (*4*) had shown that in immature pod color, red or black blotching is

[1] Received for publication May 18, 1934; issued January, 1935.

[2] Reference is made by number (italic) to Literature Cited, p. 927.

dominant over self green and that red flowers or red-veined yellow flowers are dominant over pure yellow flowers. Both flower and immature pod colors were used as a basis for the natural-crossing determinations.

Three strains of pigeonpea breeding true for the recessive pure yellow flower color and self green pod color, were planted in alternate rows with strains bearing red or red-veined yellow flowers and blotched pods. The rows with plants bearing the recessive characters were carefully rogued for any possible heterozygous types of flower and pod color the first season, insuring that the pure recessives only would be left in those rows. The plants were allowed to bloom and interpollinate freely if they would. Insects were numerous and flowers were being constantly visited by several species of the Hymenoptera group. Plants chosen at random from the rows bearing the recessive yellow flowers were harvested and the seed from each plant kept for progeny studies. Certain recessive plants were selfed by covering the unopened buds with finely woven muslin bags to determine the efficiency of this method in preventing natural crossing and to serve as a check on the open-pollinated material. The following season, progenies were grown both from the selfed and from the open-pollinated plants. The progenies from the open-pollinated plants began to show the effect of natural crossing in the many red-flowered off-types that appeared, and counts on the color of pods and flowers were made. Plants bearing flowers with the back of the standard red, or with red veins, were considered natural hybrids, while the plants with pure yellow flowers were considered as having been self-fertilized or fertilized by another member of the recessive strain. The results of this experiment are shown in table 1, progenies from the various plants, as well as the combined results for each strain, being included.

The correlation between the presence of red color in the immature pod and in the flower appeared to be so nearly perfect that the two color characteristics are reported together.

The percentage of natural hybrids, according to the data presented, is extremely high, averaging about 15 percent for the 3 strains used in this study.

Counts of the progeny of plants whose unopened flowers were covered with muslin bags gave a very few off-types which were probably natural hybrids, due undoubtedly to chance contamination by foreign pollen. The cloth bags had to be removed occasionally because of the presence of larvae of a small blue butterfly (*Lycaena boetica*) from eggs laid before the plants bloomed. Often the bees were so numerous that during the changing of bags on the flowers there was some chance for crossing unless extreme care was taken. The percentage of off-types appearing in progeny of these covered plants was extremely low, however, and many progenies showed no natural hybrids at all. The results for single-plant progenies and the combined results for all progenies in the three strains under experiment are given in table 2.

TABLE 1.—*Natural hybrids in progenies of open-pollinated plants*

Strain	Progeny no.	Plants with yellow flowers and green pods	Plants with red-veined flowers and blotched pods	Total plants	Natural hybrids
		Number	*Number*	*Number*	*Percent*
S-105	33. 117	595	147	742	19. 81
	33. 118	683	90	773	11. 64
	33. 119	761	151	912	16. 56
	33. 120	748	130	878	14. 81
	33. 121	613	167	780	21. 41
	33. 122	746	150	896	16. 75
	33. 123	323	93	416	22. 36
	33. 124	1. 433	348	1, 781	19. 54
	33. 126	701	126	827	15. 24
	33. 135	529	33	562	5. 87
	33. 136	617	50	667	7. 50
	33. 137	518	59	577	10. 23
Total		8, 267	1, 544	9, 811	15. 74
S-59	33. 127	370	66	436	15. 14
	33. 128	342	55	397	13. 85
	33. 129	370	88	458	19. 21
	33. 130	386	68	454	14. 98
	33. 131	607	78	685	11. 39
	33. 132	238	30	268	11. 19
	33. 133	275	73	348	20. 98
	33. 134	446	67	513	13. 06
	33. 138	646	88	734	11. 99
	33. 139	322	44	366	12. 02
	33. 140	419	80	499	16. 03
	33. 141	316	62	378	16. 40
	33. 142	528	85	613	13. 87
	33. 143	676	126	802	15. 71
	33. 144	594	101	695	14. 53
	33. 145	743	99	842	11. 76
	33. 146	640	130	770	16. 88
	33. 147	788	75	863	8. 69
Total		8, 706	1, 415	10, 121	13. 98
Ac. 218	32. 100–4	134	16	150	10. 67
	32. 100–9	155	35	190	18. 42
	32. 102–4	177	21	198	10. 61
	32. 102–9	168	41	209	19. 62
	32. 104–5	119	49	168	29. 17
	32. 104–10	322	92	414	22. 22
	32. 106–2	225	31	256	12. 11
	32. 106–7	269	66	335	19. 70
	32. 108–3	191	39	230	16. 96
	32. 108–8	260	30	290	10. 34
	32. 110–5	105	45	150	30. 00
	32. 110–10	302	60	362	16. 57
	33. 148	574	97	671	14. 46
	33. 149	502	74	576	12. 85
	33. 150	663	89	752	11. 84
Total		4, 166	785	4, 951	15. 86

TABLE 2.—*Natural hybrids in progenies of plants covered with muslin bags*

Strain	Progeny no.	Plants with yellow flowers and green pods	Plants with red-veined flowers and blotched pods	Total plants	Natural hybrids
		Number	*Number*	*Number*	*Percent*
S-105	33.101	150	0	150	0.00
	33.102	265	2	267	.75
	33.103	442	0	442	.00
	33.104	504	2	506	.40
	33.105	499	0	499	.00
	33.106	483	0	483	.00
	33.107	520	0	520	.00
	33.110	367	1	368	.27
Total		3,230	5	3,235	.15
S-59	33.111	458	0	458	.00
	33.113	409	0	409	.00
	33.115	316	2	318	.63
	33.116	263	0	263	.00
Total		1,446	2	1,448	.14
Ac. 218	33.1	516	2	518	.39

DISCUSSION

The results obtained in the present study indicate that there is a high percentage of natural crossing between varieties of pigeonpeas when grown in adjacent rows. The percentages of heterozygotes, or natural hybrids, in the progenies of open-pollinated pure-breeding strains as determined by color of flower and pod, range from 13.98 to 15.86 in the three strains included in the present study. This appears to be rather high for species considered to be normally self-fertilized. Shaw (*7*) in 1932 presented data to show that in India natural crossing takes place quite readily, but no percentages given were as high as those obtained in the experiments just reported. Krauss (*1*, *3*), working in Hawaii, found very little natural crossing, probably less than 1 percent. The data obtained in the present study, however, give rather conclusive evidence that a much higher percentage of natural crossing takes place when different varieties are grown in adjacent rows.

As observed by Krauss (*3*) and later by the authors, upon examination of hundreds of unopened buds, it was found that the anther sacs dehisce a day before the flower opens. In order that a great amount of natural crossing can take place, then, it would seem that there must be a differential growth rate of pollen from various sources or that some inhibiting physiological or morphological factor prevents selfing before the flower opens. Further experimentation should be carried on to determine at what stage in flowering fertilization normally takes place. Bees visit the flowers in great numbers and could readily bring about cross-fertilization, if fertilization has not taken place by the time the flower opens.

The fact that comparatively few off-types were obtained in progenies from plants artificially selfed indicates that approximate purity of strains can be maintained by covering unopened buds with muslin bags and saving seed only from such selfed flowers.

SUMMARY

Results obtained in experiments in natural crossing among varieties and strains of the pigeonpea (*Cajanus indicus* Spreng.) have been presented.

Evidence is given to show that crossing takes place freely between adjacent rows of different varieties, ranging in amount from 13.98 to 15.86 percent, with an average of 15 percent in three strains in a total population of 24,883 plants.

Progenies from plants protected from foreign pollen by covering unopened buds with muslin bags showed but 9 natural hybrids, or 0.17 percent, of a total of 5,201 plants, these few being due, probably, to faulty technic in bagging.

The amount of natural crossing which takes place between varieties under favorable conditions is great enough to necessitate the use of some definite method of plant isolation, or the covering of buds, to insure self-fertilization, in any breeding program in which the isolation and maintenance of pure strains from genetically mixed material is desired.

LITERATURE CITED

(1) KRAUSS, F. G.
1921. THE PIGEON PEA (CAJANUS INDICUS): ITS CULTURE AND UTILIZATION IN HAWAII. Hawaii Agr. Expt. Sta. Bull. 46, 23 pp., illus.

(2) ———
1927. IMPROVEMENT OF THE PIGEON PEA; GENETIC ANALYSIS OF CAJANUS INDICUS AND THE CREATION OF NEW VARIETIES THROUGH HYDBRIDIZATION AND SELECTION. Jour. Heredity 18: 227–232, illus.

(3) ———
1932. THE PIGEON PEA (CAJANUS INDICUS): ITS IMPROVEMENT, CULTURE AND UTILIZATION IN HAWAII. Hawaii Agr. Expt. Sta. Bull. 64, 46 pp., illus.

(4) MAHTA, D. N., and DAVE, B. B.
1931. STUDIES IN CAJANUS INDICUS. India Dept. Agr. Mem., Bot. Ser. 19: 1–25, illus.

(5) SHAW, F. J. F.
1930. REPORT OF THE IMPERIAL ECONOMIC BOTANIST. Agr. Research Inst. Pusa, Sci. Repts. 1928–29: 10–29.

(6) ———
1931. REPORT OF THE IMPERIAL ECONOMIC BOTANIST. Agr. Research Inst., Pusa, Sci. Repts. 1929–30: 10–32.

(7) ———
1932. THE BREEDING OF CICER AND CAJANUS. Bull. Imp. Bur. Plant Genetics, Herbage Plants [Aberystwyth] 7: 14–16, illus.

STATISTICAL ANALYSES FOR FINDING A SIMPLE METHOD FOR ESTIMATING THE PERCENTAGE HEART ROT IN MINNESOTA ASPEN [1]

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INTRODUCTION

One difficult problem of the timber estimator is to find a simple, practicable, yet reasonably accurate method of estimating the percentage of rot in standing trees. Present methods of estimating cull in aspen stands involve considerable guessing on the part of the cruiser. For instance according to one method now in use, the cruiser is directed to cull lightly if the trees are from 8 to 14 inches in diameter and free of fruiting bodies, and to cull heavily if the trees are 15 inches in diameter or over and bear fruiting bodies. How much is "lightly" and how much is "heavily"? A simple method based on quantitative data would be better. Therefore the objects of this investigation were (1) to determine the easily measured factors correlated with the percentage of rot in the dominant trees of stands of Minnesota aspen (*Populus tremuloides* Michaux), and (2) to find the most efficient factor or combination of these factors for indirectly estimating the percentage of rot in standing trees, i. e., a method that would be not only reasonably accurate but also simple and practicable.

STAGES OF ROT

Three stages of decay were described by Schmitz and Jackson (*6, p. 10*),[3] as follows:

In incipient decay the wood is faintly colored from light pink to straw brown. The intermediate stage includes all degrees of coloration from straw to chocolate brown, but the wood is apparently still hard and firm. The final stage includes all soft, punky wood irrespective of color. The classification was limited to three stages in order to allow an approximate determination of cull for certain industries utilizing aspen. For example, the excelsior industry apparently accepts and utilizes all sound, firm wood irrespective of color. The pulp and paper industry accepts, as far as we are able to determine, wood having a fairly dark color; but does not want wood having a low specific gravity. The box-shook industry apparently discriminates against the dark wood in scaling, even tho it may appear to be still quite hard and firm. If a match industry utilizing aspen wood is ever developed in Minnesota, it is not unlikely that even slight discoloration might be considered as cull. However, the practice of culling varies greatly even in the same industry. The above statements indicate the apparent trend of culling in the various industries utilizing aspen.

For the present analysis, the following classifications of rot were considered most important: Total rot, a combination of the three foregoing stages; intermediate plus final rot; and final rot alone.

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[3] Reference is made by number (italic) to Literature Cited, p. 942.

Although the major part of this investigation was confined to the total rot, in the final analysis the intermediate plus final rot was considered the most important for general use in estimating aspen stands.

BASIC DATA AND METHODS

The present investigation is based on data collected in 1926 by Schmitz and Jackson (*6*) and others. The sample consists of 77 sample plots, from one-tenth to 1 acre in area from well-stocked and understocked stands distributed throughout northern Minnesota, and tree and rot measurements on 236 sample dominant trees cut in these stands. These sample trees were cut into 8-foot bolts, and the bolt and rot diameters were measured by the methods commonly used to measure trees for the preparation of volume tables.

COMPUTATION OF TREE AND ROT VOLUME

The sample tree and rot volumes [4] in cubic feet were computed by Schmitz and Jackson (*6*) as follows: The volume of the stump and the stump rot were computed by the cylinder-volume formula; each 8-foot bolt and the rot in the bolt, if visible at both ends, by Smalian's formula; and the tip of the tree by the cone-volume formula. If the rot was visible only at one end of a bolt, its volume was computed by assuming it to be a 2-foot cylinder. Both the peeled volume of the entire stem in cubic feet and the peeled volume of the merchantable stem above a 1-foot stump to a 3-inch top were used as a basis for computing the percentage of rot. The percentage of rot in the dominant crown class of each stand was computed by dividing the total volume of rot in the sample dominants by the sum of their volumes.

This investigation was limited to the dominant trees because most of the sample trees are from this crown class, and because the dominant trees are the only ones usually cut at present.

METHOD OF ANALYSIS

The major steps in the analysis are: (1) Derivation of multiple linear equations involving different combinations of factors; (2) tests of significance to discover significant factors, and significant and efficient combinations of these factors; (3) multiple curvilinear analysis of several efficient linear equations to improve the estimates by introduction of curvilinear relationships; (4) tests of significance to find the most efficient curvilinear method.

In the linear analysis, Wallace and Snedecor's (*8*) tabular methods and their adaptations of Fisher's (*3*) methods were used not only to compute the simple, partial, and multiple correlation coefficients, regression coefficients, and standard errors, but also to test the significance of these coefficients. In addition, Fisher's z test was used to test the significance of the differences between multiple correlation coefficients and between multiple correlation indexes.

Bruce and Reineke's (*1*) method of solving problems in curvilinear correlation, the details of which will not be repeated here, was used for the curvilinear analysis. Some of their refinements were omitted, however, since it was believed that the resulting improvements would be without practical significance.

[4] It is suggested that in the future both the rot and tree-taper measurements be plotted on Reineke's tree-measurement cross-section paper. U. S. Government Printing Office Form 558a.

SELECTION OF POTENTIAL INDEPENDENT VARIABLES

Inasmuch as rot percentage is the ratio of rot volume to tree volume, it must likewise be a function of both the rot and tree dimensions. Rot diameter and length increase with time and possibly vary with site. Tree diameter and height increase not only with age, but also with an improvement in site, provided age is constant. Therefore, the following factors were selected as possible independent variables for indirectly estimating the percentage of rot: (1) Rot diameter 1 foot from the ground; (2) the depth to the rot on the average radius at 1 foot (depth to rot was used not only because of its simplicity, but also because it is a function of both rot diameter and tree diameter); (3) diameter breast high; (4) total height of the sample dominants; (5) time or age; and (6) as an index of the productivity of the environment, site index at 50 years. Different combinations of these variables were set up as hypotheses to be tested by the analysis.

REJECTION OF ABNORMAL PLOTS

Evidence of abnormal plots was revealed by a preliminary analysis To check this, the deviation from a preliminary multiple regression equation was computed for each stand. Plus or minus twice the standard error of this equation was used as the limits of rejection. Although on this basis only three plots were discarded, the definition of the curves was improved.

SIMPLE LINEAR ANALYSIS

SCATTER DIAGRAMS

The scatter diagrams in figure 1 clearly show the degree of gross relationship between rot percentage and each independent variable. In most cases, a straight line would not be a bad fit for the greater part of each variable's range. This fact gives additional weight to the conclusions drawn from the simple correlation coefficients. These diagrams also show the limited range of site index. This may explain the absence of correlation between site index and each of the other factors.

SIMPLE CORRELATION COEFFICIENTS

The simple correlation coefficients are given in table 1 not only to show the factors correlated with rot percentage, but also to show the high intercorrelation between pairs of independent variables.

TABLE 1.—*Simple correlation coefficients*

Variable	Correlation coefficients of independent variables					Site index
	Age	Diameter breast high	Height	Rot 1 foot above ground		
				Depth to rot	Diameter	
	Years	*Inches*	*Feet*	*Inches*	*Inches*	*Feet*
Diameter breast high	+0.871					
Total height	+.873	+0.809				
Depth to rot	+.537	+.706	+0.690			
Rot diameter	+.822	+.916	+.887	+0.447		
Site index [1]	*−.216*	*+.029*	*+.125*	*+.199*	*−0.019*	
Percentage of total rot [2]	+.838	+.820	+.770	+.456	+.836	*−0.114*

The coefficients in italics are not significant.

[2] In the entire stem volume.

Significance of the Simple Correlation Coefficients

To determine how much confidence to place in these simple correlation coefficients, the significance of each coefficient was tested by Wallace and Snedecor's (*8*) adaptation of Fisher's (*3*) method. For the number of degrees of freedom involved, the least significant and least highly significant correlations are +0.234 and +0.304. The least significant value (Wallace and Snedecor (*8, p. 62, table 16*)) of r corresponds to Fisher's (*3*) 5-percent level of significance and the least highly significant value to his 1-percent level.

These levels of significance correspond to 2 and 2.6 times the standard deviation in the normal curve theory of sampling.

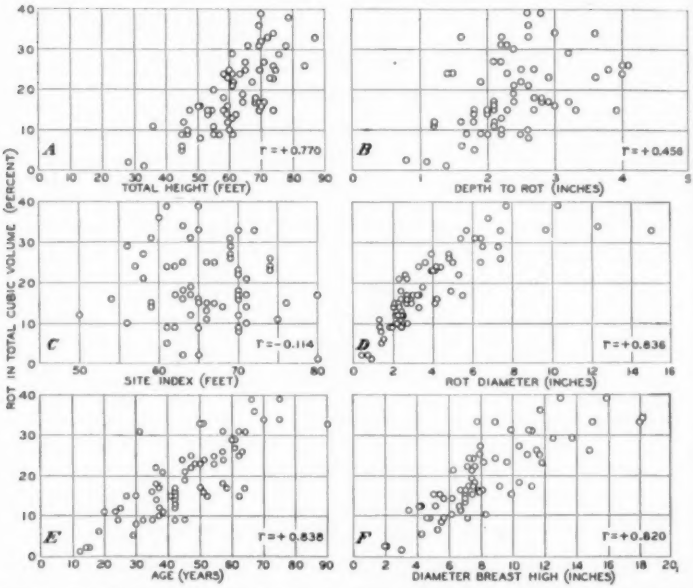

FIGURE 1.—Note high gross correlation between the percentage of total rot in the entire stem and the following factors: Total height (*A*), rot diameter (*D*), age (*E*), diameter breast high (*F*); relatively lower correlation with depth to rot (*B*); absence of correlation with site index (*C*).

Judged on this basis, age, diameter breast high, total height, depth to rot, and rot diameter are significantly and positively correlated with total rot percentage. Tests of significance also show that these independent variables, with the exception of depth to rot, are about equally correlated with rot percentage, i. e., the differences between their correlation coefficients are not significant. Although not so closely associated with rot percentage, the correlation coefficient of depth to rot is, however, still significant. Therefore it can be stated with some degree of assurance that the percentage of total rot in Minnesota aspen does increase with these factors. On the other hand, none of the site-index correlation coefficients is significant, indicating that in these data rot percentage and the other variables do not vary with site index. This is the first indication from this sample that site

index may not be very important in estimating the percentage of total rot in Minnesota aspen.

The absence of correlation between site index and several of these factors can be easily explained. Age and site index, for instance, should not be correlated if the sample plots in each age class are well distributed throughout the range of site-index classes. Diameter breast high and total height should not increase with site index unless age is kept constant, i. e., within narrow limits such as a 10-year age class. On the other hand, the absence of correlation between site index and each of the factors depth to rot, rot diameter, and rot percentage, cannot be so easily explained. Since a correlation coefficient varies with the range of the independent variable, it is quite possible that the narrow range of site index (as shown in the scatter diagram) is partly responsible for this wholesale lack of correlation with site index. Even though the limited range of site index might explain this absence of correlation, it was thought worth while to test the effect of uncontrolled age on this relationship by computing the partial correlation coefficients [5] (with the linear effect of age eliminated) between site index and each of the factors depth to rot, rot diameter, and rot percentage. These are +0.508, +0.467, and +0.165. The least significant and least highly significant values of a partial coefficient for the degrees of freedom involved are +0.236 and +0.307. Under these conditions, the correlations between depth to rot and site index, and rot diameter and site index, are significant and indicate that within an age class both depth to rot and rot diameter increase with site index. Since the coefficient, +0.165, is not significant, rot percentage in this sample does not vary with site index even though age is statistically controlled. This is also shown in table 3 by the fact that the beta regression coefficient for site index is not significant when rot percentage is expressed as a linear function of site index and age. On the other hand, the minus sign of the coefficient of site index in the linear equation, given on page 934, in which rot percentage is expressed as a linear function of site index, height, and rot diameter, indicates that under these conditions rot percentage decreases with an increase in site index.

MISCELLANEOUS CORRELATIONS

Does the percentage of heart rot vary on different soils? The answer to this question was sought in order to obtain additional light on the relationship between rot percentage and site. For this study, 35 plots with complete soil descriptions were available. Fisher's analysis-of-variance method and z test were used to test for variation between rot percentage and soils. No statistical evidence was found in the data to show that the percentage of final plus intermediate rot varies with the following soils: Sand, silt, loam, and clay. This, it would seem, is another indication that the percentage of heart rot in the dominant trees of Minnesota aspen stands does not vary with site.

Although only 25 dominant trees bearing fruiting bodies were available, it was thought worth while to find out, for example, whether or not the number and maximum height of fruiting bodies would be better indexes or rot percentage than those previously tried out.

[5] The partial correlation coefficients would also be affected by the limited range of site index, since they are a function of the simple coefficients.

For this analysis, the percentage of intermediate plus final rot in the merchantable stand was used as a basis. The least significant and least highly significant coefficients are +0.413 and +0.526. Since the coefficient +0.322, between number of fruiting bodies and rot percentage, is less than either of these, it is not significant. On the other hand, the coefficient +0.604, between maximum height of fruiting bodies and rot percentage is highly significant. Since in the major analysis rot diameter turns out to be the most important single factor, it was somewhat of a surprise to learn that rot diameter is not significant when rot percentage is expressed as a linear function of both rot diameter and maximum height of fruiting bodies. Judging from this, it might be worth while to investigate the possibilities of using maximum height of fruiting bodies for estimating the percentage of rot in those species on which fruiting bodies are common.

The simple correlation coefficients in table 1 were used to derive the multiple regression equation which follows.

MULTIPLE LINEAR ANALYSIS

MULTIPLE LINEAR REGRESSION EQUATION BASED ON THE POTENTIAL INDEPENDENT VARIABLES

The recomputed equation based on the plots remaining after rejecting abnormal plots is as follows:

$$P = -0.18 \text{ age} - 0.04 \text{ diameter breast high} - 1.10 \text{ depth to rot} + 2.35 \text{ rot diameter} + 0.50 \text{ height} - 0.37 \text{ site index} + 15.2.$$

P is the percentage of total rot in the entire stem.

The standard error of this equation is 4.2. The alienation coefficient, 0.46, shows that 46 percent of the variation in rot percentage is not accounted for by the factors considered and the inherent linear relationships of this equation.

SIGNIFICANCE OF THE MULTIPLE REGRESSION COEFFICIENTS

The relative importance of each independent variable in this equation was determined by testing the significance of the beta regression coefficients by Fisher's (*3*) t test of significance. In this test, t is the ratio of a beta coefficient to its standard deviation.

For each independent variable, the beta coefficient, its standard error, and the ratio t are given in table 2.

TABLE 2.—*Significance of regression coefficients*

Independent variable	Beta regression coefficient	Standard deviation of beta coefficient	t ratio of beta coefficient to its standard deviation
Total height	+0.67	0.18	3.7
Rot diameter	+.68	.20	3.4
Site index	−.26	.09	2.9
Age	−.31	.24	*1.3*
Depth to rot	−.09	.12	*.7*
Diameter breast high	−.02	.27	*.1*

[1] The figures in italics are not significant.

The least significant and least highly significant values (*8*, *table* 16) of t are 2 and 2.7. Judging from these criteria, total height, rot diameter, and site index are highly significant. On the other hand, diameter breast high, depth to rot, and age are not significant in this combination. Evidently the percentage of total rot in the entire stem can be estimated as closely by a linear equation involving only height, rot diameter, and site index as it can be by using all six variables. In other words, age and depth to rot can be dropped from this equation without significantly affecting its precision.

The significance of a given variable, however, is not absolute, but only relative. This is clearly shown in table 3 by the variability of t for a given factor in different combinations. For instance, when rot percentage is expressed as a linear function of tree diameter, age, height, and rot diameter, both age and tree diameter are not significant. If rot diameter is dropped, however, tree diameter and age become significant; but height loses its significance. Judging from this, the significance of a given independent variable depends not only on the size of its simple correlation coefficient, but on the combination of variables used and their intercorrelation.[6] For this reason several linear equations with different combinations of the independent variables must be set up before the most efficient combination can be discovered.

Although the linear equation determined by this test of significance will not necessarily give the most efficient curvilinear method, it is the most precise linear equation of significant variables that can be obtained from the given sample of data and the given set of potential independent variables. For this reason, it is an excellent starting point for the trial-and-error search for an efficient method.

THE TRIAL-AND-ERROR METHOD OF FINDING AN EFFICIENT METHOD FOR ESTIMATING ROT PERCENTAGE

The trial-and-error method consists of trying out different combinations of variables in multiple linear equations and noting the effect of dropping each variable in turn on the significance of the remaining variables and the standard error of the equation. In setting up these combinations in the present investigation, the most precise multiple linear equation for the data was used as a starting point. In this equation, rot percentage is a function of site index, total height, and rot diameter as follows:

$$\text{Rot percentage} = -0.25 \text{ site index} + 0.30 \text{ height} + 1.92 \text{ rot diameter} + 9.7$$

The value of t for each independent variable is, respectively, 3.1, 5.4, and 10.8, showing that site index would have the least effect on the standard error if it were dropped from this equation. Furthermore, site index for aspen is difficult to determine, since age, which is necessary for finding site index, is also difficult to determine because aspen rings are indistinct and rot is so prevalent. For these reasons, site index was the first factor to be dropped in an attempt to simplify this equation. After site index was dropped, the standard error increased from 4.2 to 4.4. Although this loss in precision is statistically significant, it was considered to be outweighed by the gain in sim-

[6] This is also evident from the formula for the standard deviation of a beta coefficient.

plicity. To simplify this equation still further, diameter breast high was substituted for total height, with the result that the standard error increased from 4.4 to 4.9. When diameter breast high was dropped, thus expressing rot percentage as a function of rot diameter alone, the standard error increased only from 4.9 to 5. In addition to these equations derived from the most precise linear equation, other promising combinations of independent variables, including those commonly used in forest mensuration, such as diameter and height, age and site index, etc., were used. These combinations with their multiple regression equation statistics are given in table 3.

TABLE 3.—*Combinations of independent variables and their multiple linear regression equation statistics used in selecting an efficient method of estimating rot percentage*

Independent variable	Ratio, *t*, of the beta coefficients to their standard deviations [1]						Standard error [2]	Alienation coefficient [3]	Multiple correlation coefficient
	Age	Diameter breast high	Total height	Site index	Rot at 1 foot				
					Diameter	Depth			
Site index, height, rot diameter			5.4	3.1	10.8		4.2	0.46	0.89
Height, rot diameter			4.6		7.3		4.4	.49	.87
Rot diameter							5.0	.55	.84
Diameter breast high, rot diameter		2.1			3.3		4.9	.54	.84
Diameter breast high, depth to rot		10.6				2.6	5.1	.56	.83
Diameter breast high, height		5.8	2.5				4.8	.53	.85
Diameter breast high, height, rot diameter		*.7*	4.1		3.0		4.4	.49	.87
Diameter breast high, height, age	2.9	2.7	*.7*				4.8	.53	.85
Diameter breast high, height, age, rot diameter	*1.8*	*.9*	2.1		3.7		4.4	.49	.87
Site, age	12.7			*1.0*			5.0	.55	.84
Site, age, diameter breast high	3.4	2.7		*.3*			4.8	.53	.85
Site, age, rot diameter	4.0			*.4*	4.3		4.5	.50	.87
Age, depth to rot	10.6					*.1*	5.1	.56	.86
Age, rot diameter	4.6				4.4		4.6	.51	.86
Age, diameter breast high	4.1	2.9					4.8	.53	.85
Age, diameter breast high, rot diameter	3.9	*.1*			3.3		4.5	.50	.87

[1] Least significant value, 2; least highly significant value, 2.7
[2] Dependent variable units. This analysis might have been carried out by a partial correlation analysis.
[3] Ratio of the standard error to the standard deviation of rot percentage, i. e., the percentage of variation still remaining after taking out the influence of the variables used as independent variables.
[4] The figures in italics are not significant.

The least highly significant multiple correlation coefficients for the given number of degrees of freedom involved and equations with 2, 3, and 4 independent variables are respectively +0.351, +0.386, and +0.413. Since all the multiple correlation coefficients are greater than +0.8, they are clearly significant; and, therefore, indicate that a functional relationship does exist between rot percentage and these different combinations.

After several combinations had been set up, it was apparent from their standard errors that several different combinations of factors would estimate rot percentage with equal precision. This, of course, made it possible not only to stress simplicity in setting up additional combinations, but also to give it considerable weight in the final choice of a method. Since the independent variables are not only about equally correlated with rot percentage but are also intercorrelated, it was possible to simplify a given combination by substituting

one variable for another without materially affecting the standard error. The number of significant variables in the most precise equation also was found helpful in setting up these combinations. Apparently the number of significant variables in the most precise equation is the maximum number of the given variables that will be significant in any combination. In this case not more than three variables were significant.

CURVILINEAR ANALYSIS

Simplicity, relative precision, and prospects of curvilinear relationships were the three factors taken into consideration in choosing the most efficient linear equations for the curvilinear analysis. On this basis, the following combinations were selected for the curvilinear analysis: Diameter breast high and total height; diameter breast high and rot diameter; diameter breast high and depth to rot; rot diameter and total height; and age [7] and rot diameter. In addition to these combinations, diameter breast high and rot diameter were used alone as independent variables. In each case the curvilinear analysis was carried through the third estimate.[8] The efficient multiple linear equations together with the statistics for these equations and the final estimating charts are given in table 4.

TABLE 4.—*Efficient multiple linear regression equations and statistics of these and the final estimating charts*

Multiple linear equation or independent variable	Standard error		Alienation		Correlation	
	Multiple linear equation	Final chart	Coefficient multiple linear equation	Index, final chart	Coefficient, multiple linear equation	Index, final chart
Rot diameter		3.7		0.41		0.91
$P=+0.27$ age $+1.56$ rot diameter $+0.4$	4.6	3.7	0.51	.41	0.88	.91
$P=+0.28$ height $+2.00$ rot diameter -5.7	4.4	3.8	.49	.42	.87	.91
$P=+0.90$ diameter breast high $+1.82$ rot diameter $+4.4$	4.9	3.9	.54	.43	.84	.90
$P=+2.67$ diameter breast high $+3.10$ depth to rot $+4.4$	5.1	4.5	.56	.50	.83	.87
$P=+1.68$ diameter breast high $+0.20$ height -6.9	4.8	4.8	.53	.53	.85	.85
Diameter breast high		5.1		.56		.83

SIGNIFICANCE OF DIFFERENT METHODS OF ESTIMATION

TESTING THE SIGNIFICANCE OF THE DIFFERENCE BETWEEN MULTIPLE LINEAR EQUATIONS

When the most efficient linear equations are being selected for a curvilinear analysis, it is helpful to know whether or not one equation based on several independent variables will give a significantly better estimate than one based on fewer and different independent variables. This can be found by testing the significance of the difference between

[7] Age was included in one combination in the hope that it would increase the precision of the method enough to warrant its inclusion in the final method.

[8] The writer can see no reason why the number of estimates through which the curvilinear analysis should be carried could not be determined by testing the significance of the differences between average residuals for consecutive estimates by Fisher's analysis-of-variance method for a unique sample.

the two multiple correlation coefficients by the following z test given by Tippett (7). The equation [9] for z is:

$$z=1.1513 \log_{10}\left[\frac{(R_1^2-R_2^2)(N-M_1-1)}{(1-R_1^2)\ (M_1-M_2)}\right].$$

R_1 and R_2 are the multiple correlation coefficients.
N is the number of observations.
M_1 and M_2 are the number of independent variables in the equations.

The least significant and the least highly significant values of z are found in Fisher's (3, table VI, p. 212) z table, for N_1 and N_2 degrees of freedom. In this case $N_1=M^1-M^2$, i. e., the difference between the number of independent variables in the equations being tested. $N_2=N-M_1-1$, i. e., one less than the difference between the number of observations and the number of independent variables in the equation with the larger value of R. If the computed value of z from the foregoing formula is not significant, the equations are equally precise. In that case, the more practical equation can be selected. Unfortunately these tests cannot be used when the independent variables are the same.

TESTING THE SIGNIFICANCE OF THE DIFFERENCE BETWEEN CURVILINEAR METHODS

A test similar to the foregoing one for linear equations would be very helpful in selecting the most efficient curvilinear method. By means of such a test, not only the significance of the difference between a multiple linear equation and its curvilinear counterpart, but also the significance of the difference between curvilinear methods could be tested. Until statisticians devise a satisfactory test for this purpose, the writer can see no reason why the following test cannot be tentatively used.[10]

Since the test for multiple correlation coefficients is nothing more than a test of the significance of the difference between variances; and, furthermore, since correlation coefficients and correlation ratios and indexes are similar statistics, the writer can see no reason why correlation indexes cannot be substituted in the foregoing formula as follows:[11]

$$z=1.1513 \log_{10}\left[\frac{(CI^2_1-CI^2_2)\ (N-M'_1-1)}{1-CI^2_1)\ \ (M'_1-M'_2)}\right].$$

CI_1 and CI_2 are the correlation indexes of the methods being compared.
N is the number of observations.
M'_1 and M'_2 are the number of independent variable terms in the expanded general functional relationship $Y=k+f\ (x)+f\ (z)....f\ (w)$, upon which this curvilinear correlation method is based.

In order to apply this test to methods of estimation obtained by the curvilinear method of analysis used in this paper, it is necessary to

[9] The writer converted the original formula from a $\log_e$ to $\log_{10}$ basis.
[10] Tipbett (7) uses correlation ratios in an analogous formula.
[11] One of the chief objections is that the curves are freehand and have not been fitted by the method of least squares. Moreover, very little appears to be known of the form of the distribution of z, computed from correlation indexes. This, of course, makes the use of Fisher's z table questionable.

determine the values M'_1 and M'_2 from the general functional equation $Y=k+f(x)+f(z)....f(w)$, upon which this curvilinear method of analysis is based. In this equation, $f(x)$, etc., is the functional relationship graphically represented by a net regression curve. But before M'_1 and M'_2 can be determined, a specific mathematical formula must be selected for each net regression curve and substituted for the terms $f(x)$, $f(z)$, etc., in the foregoing equation. For this purpose Ezekiel (*2*) has used the power series, $y=a+bx+cx^2....1x^n$.[13]

To use this series, the number of terms that will best represent the data must be determined. Ezekiel (*2*) determined this by inspection. If, however, the method of successive differences (Lipka (*4*) and Running (*5*)) is used, not only can the number of terms in the power series be more accurately determined; but, in addition, the representativeness of this form of function can be tested.

After the mathematical formulas have been selected for each net regression curve,[12] the constants M'_1 and M'_2 can be determined very easily, since they are the number of independent variable terms in the expanded function $Y=k+f(x)....f(w)$. For example, if a second-degree power series will represent the net curves, the expanded function for two independent variables, x and z, is $Y=k+(bx+cx^2)+(dz+ez^2)$. Since there are four independent variable terms in this equation, M'_1 is equal to 4. M'_2 is determined in a similar manner for the curvilinear method with which the first method is compared. The value of z is then computed from the z formula and compared with the least significant and least highly significant values of z from Fisher's z table.

SELECTION OF THE MOST EFFICIENT METHOD

The method based on rot diameter alone was selected as the most efficient, since it is not only as precise as any of the other methods considered; but, in addition, is simple and practicable.

PERCENTAGE OF ROT IN THE MERCHANTABLE STEM

In general, the conclusions drawn from this detailed analysis of the rot in the entire stem were assumed to be true for the merchantable volume. Before accepting these conclusions, however, it was considered worth while to search for other types of functional relationships that might improve the estimate. Since the dependent variable is a simple percentage, a modified percentage alinement chart seemed to have possibilities. Rot diameter and tree diameter, selected as indexes of rot and tree volume, were used as independent variables. For each independent variable, nonuniform functional scales, prepared by plotting rot volume over rot diameter and tree volume over tree diameter, were substituted for the original logarithmic scales in this percentage alinement chart. This basic chart was altered by the conventional alinement-chart volume-table procedure; and, although the analysis was carried through the fourth estimate, the improvement was not significant. Bruce (*1*) suggested plotting rot percentage over the square of the ratio of rot diameter to diameter breast high. This

[12] This method assumes that the net curves approximate the true relationship between each independent variable and the dependent variable. This is questionable when the independent variables are correlated.

[13] Double-logarithmic and semi-logarithmic cross-section paper can be used to test for simple parabolic and exponential functional relationships.

method, a slight variation of the foregoing one, likewise did not significantly improve the estimate. Consequently the method based on rot diameter alone was also considered the most efficient method for estimating the cull percentage in the merchantable stem.

TABLE 5.—*Percentage of rot in the merchantable stem* [1] *of the dominant trees in Minnesota aspen stands*

Rot diameter at 1 foot from the ground	Incipient intermediate final rot	Basis, plots	Intermediate plus final rot	Basis, plots	Final rot	Basis, trees
	Percent	*Number*	*Percent*	*Number*	*Percent*	*Number*
Under 1 inch				10		
1 inch	5	8	3	16	1	5
2 inches	12	23	6	14	2	16
3 inches	18	10	10	7	4	8
4 inches	22	9	14	8	5	10
5 inches	26	5	17	4	7	5
6 inches	29	5	21		9	2
7 inches	31	4	24	5	10	4
8 inches	33		27		12	2
9 inches	34	1	29	1	14	1
10 inches	35	3	31	2	16	4
Total		68		67		57
Standard error ..... percent		21		50		62
Improvement index [2] ..... do		48		44		44

[1] Peeled volume in cubic feet above a 1-foot stump to a 3-inch top.
[2] 100-alienation index.

The final percentages for total rot, intermediate plus final rot, and final rot alone in the merchantable stem are given in table 5 and are shown graphically in figure 2. As indicated by the improvement

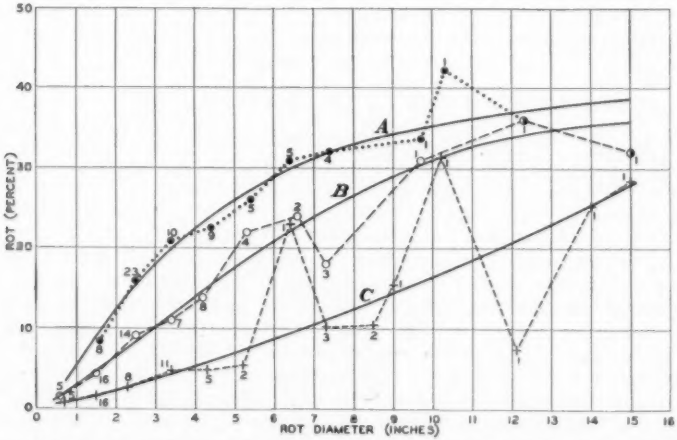

FIGURE 2.—Curves for the percentage of rot in the merchantable stem by rot-diameter classes: *A*, Total rot; *B*, intermediate plus final rot; *C*, final rot. The figure above each plotted point is the number of plots used as a basis for each raw average. The dotted and dashed lines are guide lines connecting the raw averages. The solid lines are the final curves.

indexes (100 minus the alienation index), the percentage of rot can be estimated from 40 to 50 percent more accurately by these percentages than by the use of the average rot percentage of the sample.

In spite of the 50-percent improvement, the high standard errors show that as a means of estimating rot percentage of individual stands, these percentages are still far from ideal. The best that can be said is, they are better than a mere guess. In applying these percentages, the individual stand percentages should therefore be used merely to compute the weighted average rot percentage for all the aspen stands in a given logging chance or estimating unit. Although these improvement indexes show that there is still considerable room for improvement, judging from the results of this analysis, further improvement, especially through the use of easily measured factors, will be difficult to obtain. Whenever possible, these percentages should be checked in the field by comparing the estimated rot percentage with the actual percentage obtained by the log scale. Only in this way can their representativeness and accuracy be checked and improved.

RULE OF THUMB

The percentage of intermediate plus final rot is the most important stage of rot for general use in estimating rot in cordwood stands. Since these percentages for rot diameters up to 8 inches may be represented (within the limits of accuracy of the data) by a straight line, the following simple rule of thumb can be substituted for the curve. Rot percentage=3.5 times the rot diameter. Since the average rot diameter in 96 percent of the stands measured was 8 inches or less, this rule of thumb is generally applicable.

PRACTICAL APPLICATION OF

These cull figures may be used to estimate the percentage of rot in the dominant trees of Minnesota aspen stands. They may be applied to estimates in cubic feet or cords but not in board feet. For each stand the average rot diameter at 1 foot above the ground should be determined by means of an increment borer. Usually about 25 borings are sufficient, but a larger number should be obtained if the rot diameters vary greatly. To eliminate personal bias and to insure a representative sample, the trees should be taken mechanically along the compass line. The average rot diameter to the nearest inch may be multiplied by 3.5 to obtain the percentage of intermediate plus final rot, or it may be read from table 5. These individual-stand percentages can be weighted by the gross volumes of the stands to obtain the average rot percentage for all the aspen stands in the logging chance or estimating unit. Since the standard errors of these curves are large, rot percentages should not be released for individual stands.

SUMMARY

The object of this investigation was to find a simple method of estimating the percentage of heart rot in Minnesota aspen. The conclusions reached apply only to the dominant trees. They are based on the assumption that the sample trees for a given stand are a representative sample of the dominant crown class; and that this sample of data is representative of Minnesota aspen stands.

The method used was a combination multiple linear and multiple curvilinear analysis.

All the easily measured tree and rot dimensions, in addition to age and site index, were considered as potential independent variables.

It was found that for the 25 trees bearing fruiting bodies, the number of fruiting bodies is not significantly correlated with rot percentage. For these same trees, however, the maximum height of fruiting bodies is significantly and positively correlated with rot percentage.

The percentage of total rot in the dominant trees of Minnesota aspen stands is highly and positively correlated with age, total height, diameter breast high, and rot diameter; but less closely correlated with depth to rot. Intermediate plus final rot and final rot are not so closely correlated with these factors.

For the simple two-variable relationships, there is no statistical evidence in these data to show that rot percentage varies with site index, nor that rot percentage varies with site index when the linear effect of age is eliminated. On the other hand, there is some evidence that rot percentage decreases with site index in stands having the same rot diameter and total height. This, of course, may be partly due to uncontrolled age.

Likewise there was no statistical evidence that rot percentage varies with the soil types sand, loam, silt, or clay.

Although realizing that the evidence obtained in this investigation concerning the relationship between site and rot percentage is far from conclusive, the writer feels justified in drawing the following tentative conclusion from the evidence available. For all practical purposes site need not be considered when the percentage of rot in the dominant trees of Minnesota aspen stands is estimated.

This analysis shows that the percentage of rot in dominant trees of Minnesota aspen stands can be estimated more simply and just as accurately by the use of rot diameter alone as by any other method considered in this investigation.

A rule of thumb for the percentage of intermediate plus final rot (8 inches in diameter and less) in the merchantable stem is: Rot per centage=3.5 rot diameter.

LITERATURE CITED

(1) Bruce, D., and Reineke, L. H.
1931. correlation alinement charts in forest research: a method of solving problems in curvilinear multiple correlation. U. S. Dept. Agr. Tech. Bull. 210, 87 pp., illus.

(2) Ezekiel, M.
1930. methods of correlation analysis. 427 pp., illus. New York and London.

(3) Fisher, R. A.
1930. statistical methods for research workers. Ed. 3, 283 pp., illus. Edinburgh and London.

(4) Lipka, J.
1921. graphical and mechancial computation. part ii. experimental data. 140 pp. New York.

(5) Running, T. R.
1917. empirical formulas. 144 pp., illus. New York.

(6) Schmitz, H., and Jackson, L. W. R.
1927. heartrot of aspen with special reference to forest management in minnesota. Minn. Agr. Expt. Sta. Tech. Bull. 50, 43 pp., illus.

(7) Tippett, L. H. C.
1931. the method of statistics; an introduction mainly for workers in the biological sciences. 22 pp. London.

(8) Wallace, H. A., and Snedecor, G. W.
1931. correlation and machine calculation. Iowa Agr. Coll. Off. Pub. v. 30, no. 4, 71 pp.

SOME EFFECTS OF VITAMIN-A-DEFICIENT DIETS ON REPRODUCTION OF SOWS [1]

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INTRODUCTION

It has been repeatedly shown at the California Station that sows fed certain deficient diets do not reproduce. This was true of those fed barley and salt and of those fed barley, salt, and calcium carbonate, either in the absence or presence of direct sunlight, if such feeding was begun early in life and extended over a long period. Sows on such diets came into heat and were mated to fertile boars, but no pigs were farrowed. It is well known that barley provides an excellent source of energy and phosphorus, but that its calcium content is not high. The quantity and quality of the protein of barley is not optimum for rapid growth, and the vitamin A (*4*) [2] and vitamin D [3] content of California barley is low. Since barley is used as the principal grain feed by California producers, and since many growing and mature hogs are pastured on barley stubble during the dry season in the great valleys of the State, it was decided to determine, if possible, the cause of this lack of reproduction and to find out what nutrient or accessory factor when added to barley would result in reproductiveness. Two experiments were conducted.

REVIEW OF LITERATURE

Evans (*2*) reports that a deficiency of lime in a ration had no detrimental effect on the live weight of pigs at birth. However, the appetite of the sows was materially affected, and only a small percentage of the pigs lived until weaning time. Most of the pigs died at birth or lived only from 1 to 4 days. In many cases there was no milk in the sow's udder before or after farrowing. The sows were weak and experienced considerable difficulty at parturition. The low-calcium diet fed consisted of barley meal 94 parts, maize meal 94 parts, bean meal 8 parts, blood meal 14 parts, and salt 1 part. Cod-liver oil was given as a preventive against a deficiency of vitamins A and D; and orange pulp was fed to be sure that there was no lack of vitamin C.

Davidson (*1*), using the same diet, found that there was a large number of accidents among the pigs from calcium-deficient sows. He also found that a calcium-deficient diet leads to a very considerable increase in the number of pigs born dead, the number being progressively greater in succeeding generations and litters. In the calcium-deficient group, consecutive figures of 4.8, 8.6, 28.3, and 50.0 percent of pigs born dead are most interesting. The average for the calcium-deficient group was 15.9 percent. The deficiency of calcium in the diet led to a serious reduction in the total amount of milk secreted. Fetal degeneration was excessively high among the

[1] Received for publication May 8, 1934; issued January, 1935.
[2] Reference is made by number (italic) to Literature Cited, p. 953.
[3] Unpublished data.

calcium-deficient sows, from 7 to 14 percent arising from causes other than a deficiency of vitamin E. Davidson concludes that partial fetal atrophy in sows is not due to a deficiency of protein, and that a calcium deficiency is not the major factor in producing it but it may be a contributing cause.

The effect of a diet low in vitamin A on reproduction in hogs has been discussed by Hughes, Aubel, and Lienhardt (*5*). Eight gilts were fed white corn 87 parts, or kafir 87 parts, tankage 10 parts, and bone ash 3 parts. Three would not breed and five were bred with the following results: Sow 37, aborted 6 dead pigs 86 days after breeding, 2 partially resorbed; sow 68, aborted 9 dead pigs 87 days after breeding, 1 partially resorbed; sow 67, farrowed 10 dead pigs, normal in size, no milk in her udder; sow 80, 10 normal fetuses in uterus, sow died 9 days before she was due to farrow; sow 13, 6 partly resorbed fetuses in uterus, killed 81 days after she was due to farrow. The sows and dead pigs were all examined for the porcine abortion organism. The results of the tests were negative. The authors conclude that the death of fetuses in the uterus of the sows was due to a deficiency of vitamin A in the feed of the pregnant mothers. Sows fed diets containing 10 percent of prime alfalfa meal farrowed normal litters of pigs, and those fed 5 percent of alfalfa meal showed some improvement over those fed no alfalfa, but the amount was still inadequate.

Hale (*3*) reports 11 pigs farrowed without eyeballs. The mother was a gilt and when 4 months of age was put on a vitamin-A-deficient diet. She was bred 160 days later. Thirty days after being bred she was too weak to get up and eat. For 20 consecutive days she was given 2 ounces of cod-liver oil. During the following week cod-liver oil was given every other day, after which it was discontinued. Evidence was presented to show that the condition was not a hereditary one.

Sure (*7*) induced sterility in rats, as characterized by resorption of the fetus during gestation, by maintaining them on diets deficient in vitamin A. The diets contained an abundance of vitamin E. He found opthalmia in sterile females during periods when the embryos were being resorbed.

Sherman and MacLeod (*6*) showed that a diet containing sufficient vitamin A for normal growth in rats up to nearly average adult size was not enough for successful reproduction. Seventeen females were fed a diet low in vitamin A. Only 31 young were born in this group, and none survived for more than 2 days after birth. Eleven of this group of females did not bear young. Seventeen females fed a diet higher in vitamin A bore 477 young, and of this number 264 were successfully raised and weaned. All females in this latter group produced young.

EXPERIMENTAL RESULTS

FIRST EXPERIMENT

Fifteen sows and five boars of uniform size and age were divided into five lots. There were three sows and one boar in each lot. They were kept on concrete floors, the size of the pens and houses being essentially the same. They had free access to direct sunlight. The pigs were all farrowed in March 1930, weaned May 8, and put on the experimental diets May 17. After they were weaned and

before they were placed on the experiment they were fed rolled barley, wheat middlings, and tankage, and they had access to alfalfa pasture. The following rations were fed:

Lot 1.—Rolled barley, 96½ percent; casein, 1½ percent; sodium chloride, 1 percent; calcium carbonate, 1 percent; and cod-liver oil, 2 cc per pig daily, increased to 5 cc June 24.

Lot 2.—Rolled barley, 88 percent; chopped alfalfa hay, 10 percent; sodium chloride, 1 percent; calcium carbonate, 1 percent.

Lots 3 and 4.—Rolled barley, 99 percent; sodium chloride, 1 percent.

Lot 5 (check lot).—Rolled barley, 83 percent; chopped alfalfa hay, 5 percent; tankage, 7 percent; linseed meal, 3 percent; sodium chloride, 1 percent; calcium carbonate, 1 percent.

It was believed that the pigs in lots 1 and 2 would reproduce, because in lot 1 casein, salt, calcium carbonate, and cod-liver oil were added to barley, not in large quantities but certainly in amounts sufficient for some growth; and in lot 2 alfalfa was added to barley, as well as salt and calcium carbonate. The alfalfa was a cheap source of an additional protein and vitamins A and D.

Lot 5 was fed as a check group. It was known that the diet fed this group had produced excellent growth and normal reproduction. Lots 3 and 4 were fed only rolled barley and salt during the early part of the experiment. If no reproduction resulted on this ration various nutrients were to be added one at a time until reproduction was obtained.

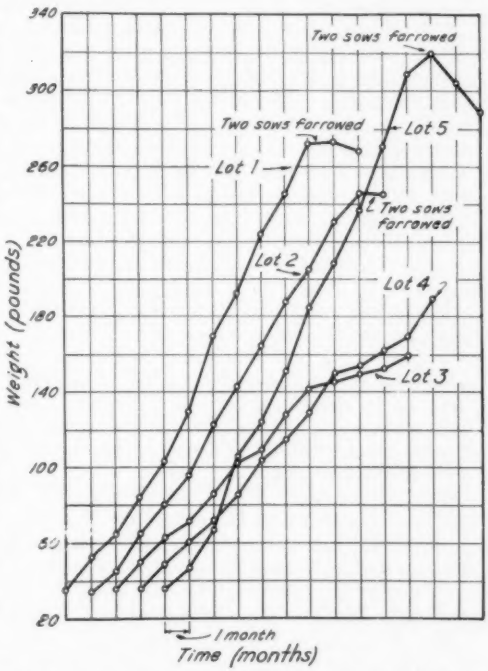

FIGURE 1.—Growth curves (average for the lots) of five lots of swine for the first 12 months of the first experiment. See text for explanation of lot numbers.

The growth curves of the animals for the first 12 months on these diets are presented in figure 1. The curves show that pigs in lots 1, 2, and 5 grew much faster than those in lots 3 and 4, and that pigs in lots 1 and 5 grew faster than those in lot 2. The ration fed to lot 5 contained more protein and the ration fed lot 1 contained less fiber than that fed lot 2.

Lot 3 was fed rolled barley and salt until February 7, 1931, when 1 percent of calcium carbonate was added and fed until February 21;

the ration was then changed to rolled barley and salt. There were no outward signs of pregnancy at this time. On March 21 the boar in the lot weighed 208 pounds. Two of the sows were doing well, the other was undersized and lacking in thriftiness. On March 28, 1 percent of calcium carbonate was added to the ration again. About 4 weeks later the pigs gave evidence of increased appetite. The sows came into heat irregularly, but there were no signs of pregnancy. The boar in this lot exhibited watery eyes, thickened eyelids, and cloudy corneas early in June. His testes were smaller than those of normal boars of his age or weight. Beginning on June 16, 10 cc of cod-liver oil was fed to each pig daily for 11 days, when the amount was increased to 20 cc. Late in the month the watery eyes, thickened eyelids, and cloudy corneas in the boar were vanishing, his testes were becoming more prominent, and he began to show an interest in the sows. Each pig in this lot gave evidence of an increased appetite. On July 4, sow 27 died. She weighed only 110 pounds at an age of about 15 months. She was not pregnant and her reproductive organs were infantile; however, her other internal organs, including the heart, lungs, liver, spleen, and kidneys, were apparently normal. Sow 14B was bred July 19 and farrowed 7 pigs (weight 15.5 pounds) on November 11. Five of the pigs died soon after birth, the other 2 died about 24 hours later. Sow 23 had not farrowed by January 13, 1932, when she was killed. She was not pregnant and her generative organs were infantile. In this group there were never outward signs of pregnancy until cod-liver oil was added to the diet in June. After cod-liver oil was given, one of the sows reproduced. The addition of calcium carbonate to the ration increased the appetite of the pigs, but apparently did not affect their reproductive organs.

Pigs in lot 4 were fed rolled barley and salt until February 7, 1931, when the ration was changed to rolled barley 98 percent, salt 1 percent, and calcium carbonate 1 percent. Like the pigs in lot 3 they showed no outward signs of pregnancy when they were 1 year of age. Beginning March 28, each pig was given 5 cc of cod-liver oil daily. This was 7 weeks after 1 percent of calcium carbonate had been included in the diet. They were fed this mixture until May 16, when the cod-liver oil was increased to 10 cc daily for each pig, and 1½ percent of casein was added to the ration. On August 14, sow 5 died (from heat prostration). Six apparently normal pigs were found in the uterus. They were estimated to be about 100 days of age, although the breeding date had not been recorded. Sow 26, bred April 28, farrowed 6 pigs (weight 16 pounds) on August 19. Sow 15, in the same lot, farrowed 7 pigs (weight 15 pounds) on December 16. They were very weak and died soon after birth.

The boar in this lot, like the one in lot 3, was sluggish, and lacked thriftiness until after he was fed cod-liver oil late in March. The addition of cod-liver oil to the diets in both groups was apparently responsible for reproduction. The sows became pregnant in about 30 days after cod-liver oil was added to their rations.

Discussion of First Experiment

The pigs in lots 1, 2, and 5 made more rapid growth than those in the other lots. They were fed rations higher in protein than those in lots 3 and 4, the calcium-phosphorus ratio was more nearly opti-

mum, and the vitamin A content of their diets was greater, until March 1931. Two sows in each of lots 1, 2, and 5 farrowed in March or early in April 1931 (table 1). These sows were 1 year of age and had been on the experiment about 10 months. There was no reproduction or outward sign of pregnancy in any of the sows in lots 3 or 4. In fact there was no sign of pregnancy in any of the sows in these lots until 30 days after cod-liver oil was added to their feed. Soon after cod-liver oil was given to pigs in these groups their appetites improved, which resulted in increased food consumption and an increased rate of gain; also, there was an immediate increase in the prominence of the testes of the boars in lots 3 and 4. All pigs had free access to direct sunlight and were fed outside; therefore vitamin D was probably not a factor. From the results of this experiment it appeared that the low concentration of vitamin A in the barley was responsible for the lack of reproduction of the sows in lots 3 and 4.

The size and condition of the various sows, boars, and young pigs on April 13, 1931, are shown in figure 2. The animals in lot 3 were thin, rough-coated, and two of them were undersized. Those in lto 5 were normal in size, thriftiness, and reproduction.

TABLE 1.—*Farrowing data for lots 1, 2, and 5 of the first experiment*

Lot no.	Sow no.	Farrowing date	Pigs farrowed	Total weight of pigs	Remarks
			Number	*Pounds*	
[1] 1	24	Mar. 9, 1931	9	24	Normal.
		Dec. 17, 1931	10	22	Individuals small, otherwise normal.
	15	Apr. 3, 1931	6	16	Weak, all died within 24 hours.
		Oct. 10, 1931	7	20	All dead except 1, which died the same day.
2	21	Mar. 3, 1931	4	11	Normal.
		Dec. 2, 1931	6	15.5	Do.
	16	Mar. 18, 1931	7	18	Do.
	14A	Feb. 18, 1932	9	23	Do.
[2] 5	16	Mar. 18, 1931	9	21	Do.
	22	Mar. 22, 1931	9	21.5	Do.

[1] Sow 25, the third sow in this lot, weighed only 20 pounds when the experiment began. She did not do well and died in October of the same year.
[2] The third sow had to be removed; she reacted to the agglutination test for swine abortion.

SECOND EXPERIMENT

At the conclusion of the first experiment the evidence strongly suggested that a low concentration of vitamin A in the barley fed was responsible for the lack of reproduction. The experiment was repeated, in its essentials, to check the results obtained. In the second series 5 groups of pigs were fed. There were 3 sows and 1 boar in each lot, divided as equally as possible in regard to condition, age, and growth. There was one difference between these pigs and those of the first experiment: They were taken directly from their mothers at weaning time and put on the experimental rations, whereas the pigs in the first trial had been weaned about 10 days before they were weighed and put on the experiment. This fact, no doubt, was responsible for the difference in the rate of growth of similar groups in the two experiments.

The plan of the second experiment differed from that of the first in one respect: Except that cod-liver oil was increased in some diets, the pigs were fed the same ration until the experiment was concluded,

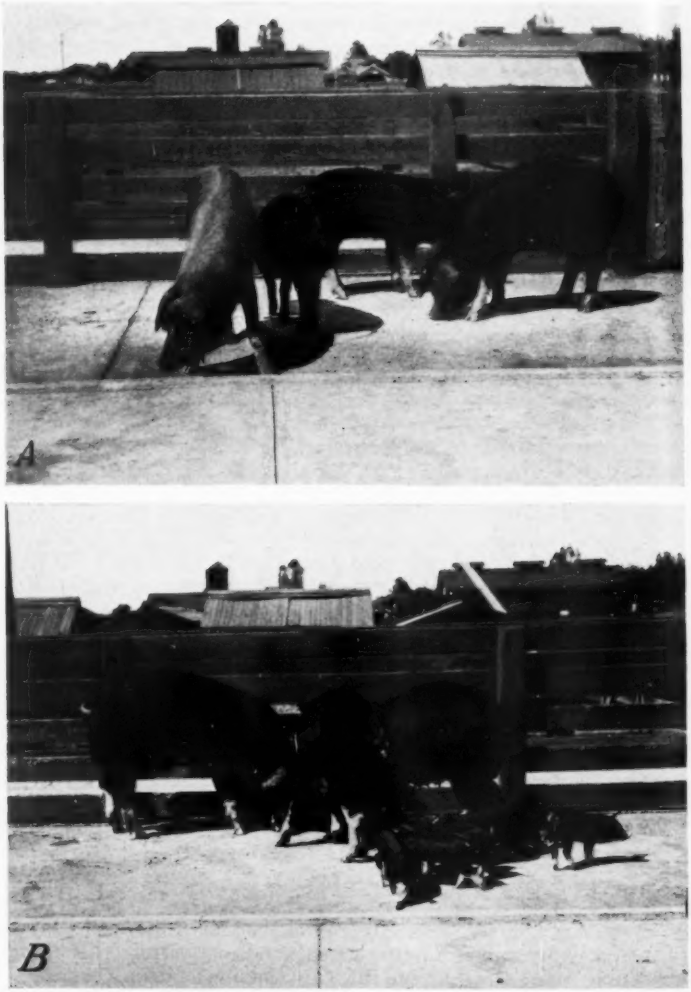

FIGURE 2.—*A*, Lot 3 of the first experiment; note lack of signs of pregnancy, thrift, or uniform growth. *B*, Lot 5 of the first experiment; these animals are well grown, uniform in size, and produced normal litters. Photographed April 13, 1931.

or until they died. The experimental animals were all farrowed the latter part of March 1931. They were divided into lots, and the experiment was begun May 23 of the same year. The following rations were fed:

Lot 1.—Rolled barley, 99 percent; sodium chloride, 1 percent.
Lot 2.—Rolled barley, 98 percent; sodium chloride, 1 percent; calcium carbonate, 1 percent.
Lot 3.—Rolled barley, 98 percent; sodium chloride, 1 percent; calcium carbonate, 1 percent; cod-liver oil, 5 cc daily per pig.
Lot 4.—Rolled barley, 96½ percent; casein, 1½ percent; sodium chloride, 1 percent; calcium carbonate, 1 percent.
Lot 5.—Rolled barley, 96½ percent; casein, 1½ percent; sodium chloride, 1 percent; calcium carbonate, 1 percent; and cod-liver oil, 5 cc daily per pig.

If the conclusions drawn from the first experiment are correct then the pigs in lots 3 and 5 of the second experiment should be expected to reproduce, while those in groups 1, 2, and 4 should not. It did not seem necessary to continue the check lot because under the same conditions they had produced normal litters of strong rugged pigs. On May 21, 1932, a year after this experiment began, the cod-liver oil fed to pigs in lots 3 and 5 was increased from 5 cc to 10 cc per pig daily.

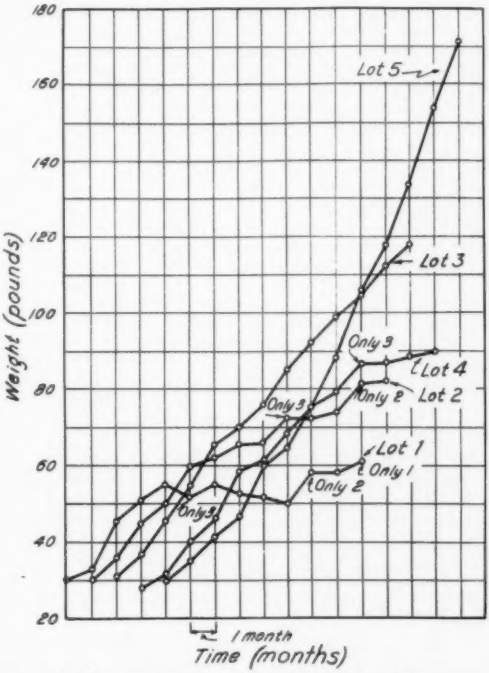

FIGURE 3.—Growth curves (average for the lots) of five lots of swine for the first 12 months of the second experiment. See text for explanation of lot numbers.

The pigs in lot 5 gained much more rapidly than did those in the other lots (fig. 3). Those in lot 3 made more uniform and much faster gains than those in lots 1, 2, and 4; and, as was expected, those in lot 4 grew faster than those in lots 1 and 2. Those fed barley without any additional protein or source of vitamin A other than that in the barley gained very slowly (lots 1 and 2). Pigs fed calcium carbonate in addition to barley and salt (lot 2) gained faster than those fed only barley and salt (lot 1).

By the end of the first year, all the pigs in lot 1 had died as a result of diet deficiencies, without ever showing signs of heat. Animals had died in lots 2 and 4. The diet fed to those in lot 4 was more liberal than that fed lot 2 but the vitamin A content was the same. It was decided to attempt to save one of the two lots. Two females still

alive in lot 2 were therefore transferred to lot 4. No individual had shown signs of heat in lot 2 up to the time it was discontinued May 21, 1932.

All sows in lot 3 reproduced (table 2), two of them in the fall of 1932 and the other in the spring of 1933. It is true that each of the sows produced only one litter each within 2 years and that the litters were not so large nor so strong as those farrowed by the sows in lot 5; however, the sows were fertile and farrowed live pigs.

In lot 5 all the sows farrowed twice within 2 years after the experiment began (table 2). The litters were satisfactory so far as number of pigs in each litter was concerned. The pigs at birth were all normal except three farrowed by sow 36, which were deformed in their hind legs.

TABLE 2.—*Farrowing data for lots 1 to 5 of the second experiment*

Lot no.	Sow no.	Farrowing date	Pigs farrowed	Total weight of pigs	Remarks
			Number	*Pounds*	
[1] 1	35		0		Paralyzed Sept. 19, 1931, killed Sept. 25 (vertebra found fractured).
	26		0		Died Mar. 27, 1932, of pneumonia, weight 46 pounds.
	52		0		Died May 9, 1932, of pneumonia. Boar 59 died Feb. 1, 1932, weight 38 pounds.
[2] 2	25		0		Died Mar. 12, 1932, of pneumonia.
	24		0		Transferred to lot 4, May 21, 1932.
	54		0		Do.
					Boar 60 died Dec. 19, 1931, of pneumonia.
3	22	Oct. 30, 1932	2	6	Normal.
	34	Nov. 14, 1932	1	2	Pig found in morning partially consumed by sow; probably more pigs were farrowed but not found.
	58	May 5, 1933	7	18	Small, weak, otherwise normal.
[3] 4	63		0		Died Jan. 27, 1932; very thin, hair curly.
	53		0		Died Apr. 30, 1932; very thin.
	61		0		Killed Apr. 1, 1933 (see discussion of table).
	24		0		Killed Apr. 10, 1933 (see discussion of table).
	51		0		Killed Apr. 13, 1933 (see discussion of table).
					Boar 29 died Oct. 22, 1932, of pneumonia. Another fertile boar substituted in August.
5	36	July 29, 1932	6	15	3 normal, 3 pigs with deformed hind legs.
		Dec. 22, 1932	6	17	Normal.
	62	Aug. 7, 1932	10	21	All alive except 1 at birth, small and weak.
		June 3, 1933	10	22	Normal, though small.
	31	Sept. 16, 1932	5	12.5	Do.
		May 6, 1933	7	21.0	Normal.

[1] There were no signs of heat in any of the sows in lot 1 at any time.
[2] There was no evidence of heat in any of the sows of lot 2 during the year. Lot 2 was discontinued May 21, 1932, and 2 sows transferred to lot 4.
[3] Sows in lot 4 finally came in heat but no pigs were ever farrowed.

Two sows in lot 4 died before the experiment had been in progress a year, and the boar died in October 1932. He began to lose weight rapidly in August, when he was taken out and another boar of the same age substituted for him. Since no signs of heat had ever been manifested by the sows in lot 2, and since they were far below normal in growth and thrift, two of them were transferred to lot 4, on May 21, 1932. Late in the fall it was noticed that the sows came into heat and that copulation took place, but the sows did not become pregnant. Early in November, sow 61 developed difficulty in walking. She turned her head from time to time and seemed to lack a normal sense of equilibrium. On January 30, 1933, she was still walking with

difficulty and had become phlegmatic. Her hair was long, dry, and curly, and she seemed at least partially blind. One day she was found lying flat on her side and kicking her legs violently. She became worse, stood with her back down and tail held straight, her eyes were dull, and at times she was unconscious of the presence of food. She would often spend several minutes attempting to rise, and after getting up she would stagger to the wall for support and would sometimes fall. At times she appeared to be totally blind.

By the 1st of March 1933, the other two sows in lot 4 exhibited the same symptoms though not to the same extent. They showed definite signs of vitamin A deficiency, as described by Hughes, Aubel, and Lienhardt (*5*). Their average weight was about 200 pounds. All had come into heat though none had reproduced. They were 2 years of age, and it was decided to wait until they came in heat, breed them to a boar known to be fertile, wait for ovulation, then slaughter them and examine the generative organs for signs of the beginning of ovulation. Sow 61 was bred, and 4 days later (Apr. 1, 1933) was slaughtered. Sow 24 was bred, and slaughtered 4 days later (Apr. 10, 1933). Sow 54 did not come into heat. On April 8 it was noticed she appeared to show signs of pregnancy, but 5 weeks later she exhibited no more signs of pregnancy than she had early in April, and she was slaughtered.

At autopsy the following conditions were found:

Sow 61.—Uterus and vagina apparently normal. Large serous cyst on the left oviduct. Oviduct about 2 inches in diameter and filled with yellowish fluid. Right oviduct cystic and walls thickened. Ovaries apparently nearly quiescent. A few corpora vestiges and one fairly large follicle in one ovary; several follicles about 0.5 cm in diameter in the other.

Sow 24.—Ovaries normal, no signs of recent ovulation.

Sow 54.—Eight brownish-red corpora in one ovary and 5 in the other. Brown liquefied mass of meconium in uterus, with evident resorption of fetuses. Many follicles present about 5 mm in diameter.

Discussion of Second Experiment

In the second experiment, as would be expected from the results of the first, the sows in lots 3 and 5 reproduced. The sows in the other groups did not. One sow in lot 4 ovulated, became pregnant, and the fetuses were being resorbed when the sow was slaughtered. The only difference between the diets of lots 4 and 5 was that cod-liver oil was given to the animals in lot 5, while those in lot 4 received none. Within 2 years after the experiment began each sow in lot 5 had produced two litters of live pigs, whereas the sows in lot 4 did not reproduce. All the sows in lot 3 farrowed live pigs. These sows were fed like those of lot 5 except that they received no casein in their diet. The pigs in lot 4 were fed casein but no cod-liver oil. It seems apparent, therefore, that cod-liver oil contained something which was responsible for the reproduction obtained in lots 3 and 5 It hardly seemed possible that vitamin D was a factor since all animals had had free access to direct sunlight. However, in order that rickets or osteoporosis might be eliminated as complicating factors, the total ash of the humerus of sow 61 (lot 4) was determined. It was found to be 52 percent. The vitamin A content of the liver of the same sow was determined by the antimony trichloride method, with totally negative results. There was, therefore, no storage of vitamin A in the liver of this sow.

SUMMARY OF RESULTS OF THE TWO EXPERIMENTS

The results of the two experiments are summarized in table 3.

TABLE 3.—*Summary of the results of the 2 experiments*

Diet	Pigs in each group	Sows	Boars	Sows that reproduced	Litters farrowed	Condition of pigs at farrowing
	Number	*Number*	*Number*	*Number*	*Number*	
Barley and salt	12	9	3	0	-------	
Barley, salt, and caclium carbonate	12	9	3	0	-------	
Barley, salt, calcium carbonate, and casein	6	5	1	[1] 0	-------	
Barley, salt, calcium carbonate, and cod-liver oil.	12	9	3	7	7	Weak.
Barley, salt, calcium carbonate, cod-liver oil, and casein.	8	6	2	[2] 5	10	7 strong litters, 3 weak litters.
Barley, salt, calcium carbonate, and chopped alfalfa hay.	4	3	1	3	4	Normal.
Barley, salt, calcium carbonate, chopped alfalfa hay, tankage, and linseed meal.	3	2	1	2	2	Do.

[1] There was pregnancy and resorption in the case of 1 sow.
[2] 1 sow in this group died.

SUMMARY

Details of two experiments covering a period of 3 years have been presented. The results obtained in the two were similar. No reproduction resulted when the following diets were fed from the time the pigs weighed about 40 pounds: Barley and salt; barley, salt, and calcium carbonate; barley, salt, calcium carbonate, and casein. Sows of similar weights, age, and breeding did produce when fed: Barley, salt, calcium carbonate, and cod-liver oil; barley, salt, calcium carbonate, and chopped alfalfa hay; barley, salt, calcium carbonate, casein, and cod-liver oil; barley, salt, calcium carbonate, chopped alfalfa hay, tankage, and linseed meal.

Two lots of sows fed barley and salt for nearly a year did not reproduce, even when calcium carbonate was added. The sows in both these lots farrowed litters when cod-liver oil was given to one lot and when cod-liver oil and casein was given to the other.

Barley, when fed as the only source of vitamin A, did not contain enough of this vitamin for normal reproduction in the pig.

Somewhat better results were obtained when an additional protein (casein) as well as additional vitamin A, was added to a barley, salt, and calcium carbonate diet.

Pigs fed barley, salt, calcium carbonate, and casein had all the outward symptoms of a deficiency of vitamin A.

The addition of calcium carbonate to a barley and salt diet resulted in an increased appetite, and rate of gain when fed to pigs in these studies.

LITERATURE CITED

(1) DAVIDSON, H. R.
1930. REPRODUCTIVE DISTURBANCES CAUSED BY FEEDING PROTEIN-DEFICIENT AND CALCIUM-DEFICIENT RATIONS TO BREEDING PIGS. Jour. Agr. Sci. [England] 20: [233]–264, illus.

(2) EVANS, R. E.
1928. PROTEIN AND MINERAL METABOLISM IN PREGNANT SOWS ON A NORMAL OR HIGH CALCIUM DIET COMPARED WITH A CALCIUM-DEFICIENT DIET. Jour. Agr. Sci. [England] 19: [752]–798, illus.

(3) HALE, F.
1933. PIGS BORN WITHOUT EYEBALLS. Jour. Heredity 24: 105–106, illus.

(4) HUGHES, E. H.
1933. VITAMIN A CONTENT OF BARLEY. Jour. Agr. Research 47: 487–494, illus.

(5) HUGHES, J. S., AUBEL, C. E., and LIENHARDT, H. F.
1928. THE IMPORTANCE OF VITAMIN A AND VITAMIN C IN THE RATION OF SWINE, CONCERNING ESPECIALLY THEIR EFFECT ON GROWTH AND REPRODUCTION. Kans. Agr. Expt. Sta. Tech. Bull. 23, 48 pp., illus.

(6) SHERMAN, H. C., and MACLEOD, F. L.
1925. THE RELATION OF VITAMIN A TO GROWTH, REPRODUCTION AND LONGEVITY. Jour. Amer. Chem. Soc. 47: 1658–1662, illus.

(7) SURE, B.
1928. DIETARY REQUIREMENTS FOR FERTILITY AND LACTATION: A DIETARY STERILITY ASSOCIATED WITH VITAMIN A DEFICIENCY. Jour. Agr. Research 37: 87–92, illus.

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